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3D-printed lattice structures for next-generation orthopaedic implants: Considerations on design and post-processing for biomechanical performance and osteoconductivity

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Declaration of originality

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Abstract

Additive manufacturing (3D printing) has enabled the fabrication of joint replacement implants which incorporate porous lattice structures to promote bone in-growth for strong biological fixation after surgery. To meet load-bearing requirements and reduce the risk of aseptic loosening, an ideal lattice should combine high fatigue strength, bone-matching stiffness, together with a micro-architecture and surface finish that benefit the formation of mature bone tissue on implant surface.

In this direction, this thesis comprised of four studies aiming to: (a) develop a parametric model for designing lattices with biomimetic mechanical properties and micro-architecture, (b) assess the impact of lattice micro-architecture (topology) on mechanical behaviour across different specimen sizes, (c) tailor lattice design parameters and (d) explore post-processing methods to enhance fatigue strength and drive in vitro bone tissue formation (osteogenesis).

Stochastic lattices with stiffness ranging from 0.1 to 8 GPa were developed by varying nodal connectivity, strut density and thickness. A key finding revealed that increasing connectivity induces a stretch-dominated behaviour, improving fatigue strength by up to 60% compared to bend-dominated low-connectivity lattices. Moreover, the bend or stretch dominated behaviour was found to control the stiffening mechanism of lattices depending on aspect ratio and specimen size.

From a biological perspective, high connectivity emerged as a critical parameter for tissue growth and maturation owing to the high surface area and multiple concavities introduced around the nodes. Additionally, struts with thickness similar to natural trabeculae (200–300 μm) provided a perfect balance of high surface area-to-volume ratio, essential for osteogenesis, and mechanical strength, for load-bearing capacity. Finally, a chemical-electrochemical surface treatment to smoothen lattice surface was found to improve fatigue strength and promote osteogenic differentiation.

Collectively, this work presents a holistic methodology for the development of lattices with high design flexibility, tailored mechanical properties and topological characteristics optimised to offer support mature bone tissue formation.

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List of Symbols

Greek letters

δ_p	Point distance during additive manufacturing
δ_t	Laser exposure during additive manufacturing
ΔH	Specific enthalpy
ε	Engineering strain
θ	Strut inclination angle with respect to build plate
ζ	Aspect ratio
ρ	The apparent density of a lattice structure
ρ_s	Density of solid material
σ	Engineering stress
$\sigma_{f,N}$	Fatigue strength at N cycles
σ_{pl}	Plateau stress
σ_u	Ultimate compressive strength of a lattice structure
σ_y	Yield of a lattice structure under compression
σ_{ys}	Yield of solid material under compression
Φ	Laser spot diameter

Latin letters

A	Absorptivity of solid material
d	Strut density
D	Thermal diffusivity of solid material
d_p	Pore size
E	Elastic modulus of a lattice structure
E_s	Elastic modulus of solid material under compression
ED	Volumetric energy density
f	Frequency
h	Hatch spacing during additive manufacturing
j	Number of nodes
l	Unit cell edge length in a periodic lattice structure
L	Strut length
M	Maxwell number for frame stability criterion

n	Sample size
N	Samples population size
n_1	Power index in Gibson-Ashby model for compressive strength
n_2	Power index in Gibson-Ashby model for elastic modulus
R_a	Arithmetic average roughness of 2D profile
S_a	Arithmetic average roughness of 3D surface
S_z	Maximum peak-to-valley roughness of 3D surface
s	Number of struts
t	Strut thickness/diameter
t_s	Layer thickness during additive manufacturing
v	Laser scan speed during additive manufacturing
Z	Nodal connectivity

List of Abbreviations

μCT	Micro Computed tomography
ALP	Alkaline Phosphatase
AM	Additive Manufacturing or Additively Manufactured
CAD	Computer-Aided Design
CP-Ti	Commercial Pure Titanium
BCC	Body-Centred Cubic
BMP-2	Bone Morphogenetic Protein 2
DIC	Digital Image Analysis
ECM	Extracellular Matrix
EDM	Electrical Discharge Machining
ELISA	Enzyme-linked immunosorbent assay
FCC	Face-Centred Cubic
FBS	Foetal Bovine Serum
FGF-2	Fibroblast growth factor 2
HIP	Hot-Isostatic Pressing
hMSC	Human Mesenchymal Stromal Cells
HSC	Hematopoietic Stem Cells
IGF-1	Insulin-like Growth Factor 1
IFN-γ	Interferon γ
IL	Interleukin
MEM	Minimum Essential Medium
PBS	Phosphate-Buffered Saline
PBF	Powder Bed Fusion
RT-qPCR	Real-Time Quantitative Polymerase Chain Reaction
SEM	Scanning Electron Microscopy
SOST	Sclerostin
TGF-β	Transforming Growth Factor β
TNF	Tumor Necrosis Factor
VEGF	Vascular endothelial growth factor

1. Introduction

Natural cellular materials, like trabecular bone, cork, balsa wood, sponge, and coral, are known for their highly efficient design, offering high compressive strength with low weight and stiffness. This has led to the development of man-made cellular materials (porous structures) that exhibit unique properties while made with established engineering materials like metals with completely different properties. Synthetic porous structures are now widely used across diverse applications that require high specific strength (e.g., lightweight structures in the automotive and aerospace industries), energy absorption capacity (e.g., cushioning), high surface area (e.g., insulation and heat exchangers), and biomimetic morphologies (e.g., tissue engineering scaffolds) (1,2). The combination of load-bearing capacity, low stiffness, and biomimetic architecture constitute synthetic porous structures particularly attractive in orthopaedic surgery when used as part of implants (e.g., for joint arthroplasty or spinal fusion) or as grafts (e.g., for filling osteotomies or bone defects caused by trauma, tumour, or infection).

In regard to joint arthroplasty, surgical procedures, such as knee, hip, elbow replacements, traditionally relied heavily on acrylic cement to provide stability and fixation when an implant was placed inside the bone. This technique remains popular among surgeons due to its cost-effectiveness and satisfactory survivorship (3), however, there are major drawbacks associated with cemented implants, such as extended operation time, cement fragmentation, aseptic loosening, and long-term foreign body complications. Cement may degrade over time provoking implant loosening and requiring a revision surgery (4). In other cases, there are concerns about post-operative cardiovascular complications such as vasodilation and inflammation of the implant site associated with patients' bone quality and age, or excessive use of cement (5).

Bone is a tissue with high regenerative capacity manifested as a healing response after injury, but also as continuous remodelling throughout adult life (6). During remodelling, bone adjusts its mass and architecture daily to meet altering mechanical needs and repair any damages in its matrix preventing the accumulation of old or damaged bone (7). This has led to the rise of cementless implants, which rely on the biological healing capacity of the patient's bone. Owing to the cement's absence, intraoperative and short-term post-operative implant fixation relies on a tight press-fit of the implant inside the host bone. While the use of screws or design features like pegs and keels provide the necessary implant stabilisation depending on the anatomical site (8–10). In addition, roughened surfaces generated through grit blasting (11) or by the attachment of metallic particles (12), beads (13) and fibre meshes (14) on implant surface not only aid fixation by increasing friction with bone (15), but they also provide a surface texture that favours bone to attach and grow directly on the implant surface forming a long-term biological fixation (a process termed osseointegration) (16).

In this context, cemented and cementless approaches appear to exhibit contrasting trends: cemented fixation offers a robust short-term solution with a risk of degradation over time, whilst cementless fixation banks on the longevity of the bone-implant interfacial connection. Cementless fixation has become increasingly popular for treating young and active patients who exhibit higher than average failure rates with cement fixation (17). Moreover, cementless fixation has been shown to outperform cemented fixation in terms of survivorship in morbidly obese patients, who are in general more prone to osteoarthritis and more likely to need joint arthroplasty (18).

Still, the abovementioned cementless solutions display significant limitations. First, they are characterised by low porosity, where osseointegration is limited at the interface between bone and implant (bone on growth) leading to often insufficient strength for withstanding large and repeated loads (19). Second, even in the case of strong biological fixation, the rough "coating" layer is usually not strongly attached to the implant surface, and under the effect of repeated loading it can result in delamination/debonding leaving the coating layer only attached to the bone site (20,21). Third, these implants typically constitute solid metallic parts made of titanium or cobalt-chrome alloys that are much (10 to 100 times) stiffer than natural bone. However, bone remodelling and the deposition of new bone matrix depend on the mechanical signals that bone receives daily (Wolff's law) (22). Thus, under the presence of an overly stiff implant, the bone will remain inactive (stress-shielding), leading to the loss of its mass, even bone necrosis and peri-prosthetic fracture (23). All cases eventually result in aseptic loosening which remains a leading cause of failure in (primary) cementless surgeries (24).

Joint replacement implants were historically fabricated using machining, forging, or casting. However, modern implants can now be made using additive manufacturing (3D printing), enabling the integration of complex 3D porous structures on implants with intricate shapes. Made using the same metallic materials, these structures can be designed to combine high strength with low stiffness, for withstanding physiological loads while allowing active bone remodelling. Additionally, contrary to previous cementless surfaces, additively manufactured porous structures exhibit high porosities with distinguishable 3D pores as in trabecular bone. Essentially, they serve as scaffolds that mimic the trabeculae network enabling cells from the host bone to migrate and form new bone extracellular matrix (ECM) inside the porous structure (16,25). Osseointegration is now referred to as bone in-growth and leads to a mechanically superior biological fixation. Moreover, this 3D porous architecture plays a vital role in creating an environment conducive to vascularization and nutrient exchange, essential for the maturation and preservation of the newly formed tissue (26,27). Last, the advancement of additive manufacturing enables the incorporation of screw-like (28) or anchoring (29) features for enhanced initial fixation.

This new generation of orthopaedic implants holds promises for enhanced osseointegration and reduced revision rates, allowing patients to live an active life after surgery. However, currently available additively manufactured porous implants still face considerable limitations. First, they lack mechanical strength compared to solid implants, often leading to catastrophic failure of the implant (30). Second, the porous architectures aim to mimic trabecular bone, however, there are many occasions where they induce insufficient or premature (fibrous) bone in-growth, leading to loosening (31). These long-lasting challenges do not allow the adaptation of fully porous implants that will restore physiological loading in clinical practice, while available data suggest that porous and beaded implants still exhibit comparable survivorship (32).

Musculoskeletal disorders like bone fractures and large bone defects, as well as degenerative diseases like osteoporosis and osteoarthritis, are prevalent health conditions and one of the primary causes of disability worldwide, mainly due to ageing populations and the increasing incidence of osteoarthritis which currently affects over 7% of the global population (33). Despite the effectiveness of initial surgical procedures, the rate of revision surgeries especially for young and active patients (younger than 60 years old) is more than 35% (34). These outcomes not only harm patients' lives but pose a huge economic burden on national healthcare systems as revision surgeries require longer hospitalisation and higher costs than the initial surgeries (35).

Using additive manufacturing, large-scale porous implants with complex shapes, a variety of sizes and bespoke properties can be developed. This thesis aims to address central and still-existing challenges towards this direction. More flexible approaches for the design of porous structures are needed to

enable their application in a broad range of anatomic sites. This should be accompanied by a deeper understanding and a more accurate prediction of structures' mechanical behaviour across different scales. Last, apart from tailored mechanical properties, next-generation porous implants need to combine architectural and material characteristics that will impel mature bone formation for stronger biological fixation.

2. Background

This first chapter provides an overview of bone biology, the process of osseointegration, cellular materials and the use of additive manufacturing for the development of synthetic porous structures. It concludes with critical remarks about the use of porous structures for bone regeneration.

2.1. Bone biology

The development of devices for effective bone regeneration presupposes a good understanding of bone's macroscopic structure and mechanical properties as well as its microscopic building units (cells) that orchestrate its function.

2.2.1. Hierarchical structure

Bone tissue has a complex macroscopic structure optimised for strength, flexibility, and load absorption. It is distinguished into two main regions that further demonstrate a complex sub-hierarchy. The two main regions are the cortical (compact) and cancellous (trabecular) types of bone (36). The former constitutes the outer dense layer of all bones, accounting for 80% of the total bone mass in the adult skeleton, with a primary structural and protective role that provides resistance to external multiaxial loading. The latter constitutes the inner part of the bone, which is highly porous, allowing for shock absorption, the distribution of external compressive loads, and the transportation of nutrients, minerals, and cells within its pores. Both types of bone exhibit gradient levels of porosity, they are highly vascularised and formed from the same compounds. In fact, bone is a composite material made up of an organic matrix primarily consisting of type I collagen, which accounts for approximately 40% of its volume. This collagen matrix is interspersed with mineral crystals, mainly composed of non-stoichiometric calcium hydroxyapatite, which makes up about 45% of the volume. The remaining 15% is comprised of water, either bound to the collagen or present within the empty spaces (37).

The formation of bone matrix begins at the nanoscale with the synthesis of amino acids, which are the building blocks of proteins, and bond sequentially to form polypeptide chains. About 85-90% of bone protein consists of collagen type I, a molecule formed by a triple helix of such amino acid chains. At the sub-micron-level, the bone matrix is composed of collagen fibres which is an assembly of numerous collagen molecules crosslinked with mineral crystals (**Figure 2.1a**). Multiple collagen fibrils are then organised and assembled forming larger collagen fibres. This basic unit provides the bone matrix with high mechanical properties such as tensile strength and viscoelasticity (36,38).

Mineralised collagen fibres are parallelly arranged forming sheets called lamellae (3-7 microns thick) which then form the cortical and trabecular bone. In other instances, collagen fibres can be disorganised, exhibiting random orientations, termed woven bone (36). The disorganised arrangement of collagen fibres makes woven bone mechanically weaker than lamellar. However, woven bone constitutes an early stage of the bone matrix which is formed during foetal development or fracture repair. Essentially, woven bone constitutes a temporary scaffold to guide the deposition of mature lamellae bone (39).

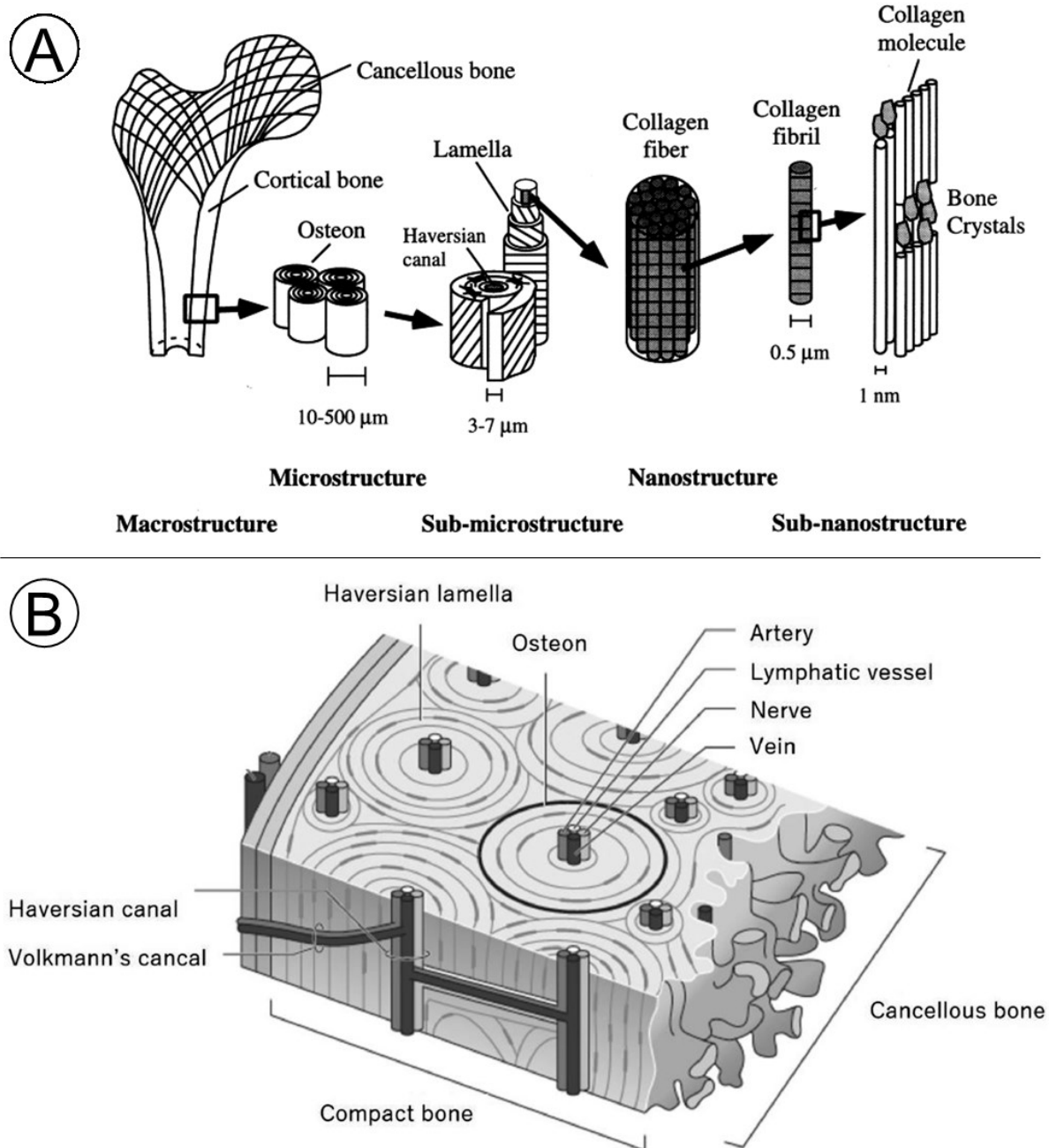


Figure 2.1. Sketches demonstrating the hierarchical structure of bone. (A) Breakdown of bone's building units. Figure reproduced from (36) with permission from Elsevier (License No. 5974990665955). (B) The microanatomy of cortical bone. Figure reproduced under CC BY license from (40).

In cortical bone, multiple lamellae form concentric layers around a central canal (i.e., the Haversian canal), this cylindrical arrangement exhibits a diameter between 200-250 μm and is known as the osteon or the Haversian system (36) (**Figure 2.1b**). Cortical bone is formed by parallelly arranged osteons, with distinct boundaries defined by cement lines. The Haversian canals run longitudinally through the bone and enclose blood vessels, nerves, and lymphatic vessels. Additional canals (the Volkmann's Canals) are oriented transversely to osteons and act as links between the Haversian canals inside the bone and larger arteries, veins, nerves, and lymphatic vessels outside the bone (40). These interconnected canal

networks are critical for supporting bone remodelling by delivering nutrients and oxygen to cells inside the bone and for removing waste products.

The trabecular bone consists of a network of interconnected beams/rods and plates, called trabeculae, which are formed by parallelly oriented lamellae, resulting in a thickness of about 50-300 μm (36,41). The space between trabeculae has been found to range between 300 – 2000 μm depending on the anatomical site, age, sex, and health condition (42,43) and is filled with red bone marrow and blood vessels (44).

Although the configuration of lamellae in cortical and trabecular bone exhibits differences, osteons and trabeculae play a central role in the mechanical response of each bone type. The elastic modulus of osteons has been found to range between 20 and 30 GPa (45), while the one of single trabeculae between 10 and 20 GPa (41). While the properties of their forming units might be comparable, the trabecular and cortical bone exhibit critically different macroscopic mechanical properties, primarily associated with differences in porosity.

2.2.2. Macroscopic mechanical properties

In humans, trabecular bone exhibits a high porosity, ranging from 50% to 90%, with an average density of around 0.2 g/cm^3 . In contrast, cortical bone is much denser, with porosity between 3% and 12%, and an average density of 1.80 g/cm^3 . This results in a wide range of elastic moduli, being 0.05 – 5 GPa in trabecular bone, and 7 – 30 GPa in cortical bone (46,47).

Bone properties exhibit noticeable differences depending on age, sex, nutrition, activity level, and bone health (48). In addition, they vary depending on the anatomical site, for example, bone with the same density demonstrates a higher apparent modulus in the tibia compared to the vertebrae or femoral neck. Inter-site differences, with inter-site differences reaching 65% (47). Last, the trabecular and cortical bones exhibit local differences in the micro-architecture and porosity. This is particularly apparent in trabecular bone, resulting in significant deviation in the mechanical properties even within the same bone (41), with intra-site differences reaching 50% (47).

2.2.3. Cell populations

Apart from its apparent structural and protective role for the body and organs, bone also serves as a reservoir for minerals (calcium, phosphate, magnesium etc. that are vital for the function of multiple tissues such as muscles, skin), blood and immune cells that are supplied in circulation through the bone marrow. Hence, bone hosts a diverse population of cells ranging from multipotent to function-specific unipotent cells. The majority of unipotent cells found in bone are derived from mesenchymal stem/stromal cells (MSC) and hematopoietic stem cells (HMC) which in turn are found in bone marrow, peripheral blood, and adipose tissue (49). These stem cells contribute to the formation and maintenance of bone, cartilage, and vasculature tissue inside the bone complex.

The major cells found in bone are the osteoblasts, osteoclasts, osteocytes, and lining cells.

Osteoblasts originate from mesenchymal stem cells, which differentiate into osteoprogenitor cells (pre-osteoblasts) before maturing into osteoblasts. This differentiation is initiated by various growth factors and hormones that activate key signalling pathways like the Wingless and Int-1 (Wnt), the BMP-Smads (bone morphogenetic and Smad proteins), and IGF-1 and 2 (insulin-like growth factors 1 and 2) pathways (50). The differentiation of MSCs to the osteogenic lineage is driven by the expression of critical proteins, primarily Runt-related transcription factor 2 (RUNX2), which is required for the initial osteogenic commitment, and Osterix, which promotes further maturation of pre-osteoblast and differentiation into osteoblasts (51).

Osteoblasts are cuboid-shaped cells with the primary function of forming new bone, which is done in two stages: the deposition of organic matrix and then its mineralisation. In the first stage, osteoblasts secrete the ECM which is composed of collagen type I, proteoglycans (such as biglycan and decorin), and non-collagenous proteins (including osteonectin, bone sialoproteins, osteopontin, and osteocalcin). To initiate the mineralisation, proteoglycans help bind and sequester calcium and phosphate ions from the surrounding environment and enzymes like alkaline phosphatase (ALP) create nucleation sites for the crystallisation of calcium phosphate into hydroxyapatite, the main mineral form found in bone (52). This mineralisation process is further regulated by proteins like osteopontin and osteocalcin, which help ensure the proper deposition of hydroxyapatite crystals within the collagen matrix. After enough hydroxyapatite has successfully been embedded within the ECM, the mineralisation process is completed, and osteoblasts reduce their activity. During this final phase, osteoblasts undergo apoptosis (programmed cell death) or transition into other cell types to maintain bone homeostasis.

A portion of osteoblasts will become bone lining cells, which remain on the bone surface. These cells play a crucial role in regulating calcium and phosphate exchange and are involved in the activation of future remodelling cycles. Lining cells are typically inactive but can reactivate when needed for bone maintenance or repair. Another fraction of osteoblasts become osteocytes, the most abundant cell type in mature bone. Osteocytes are branched cells embedded within the newly formed mineralised matrix, occupying small spaces called lacunae. From their position, osteocytes can communicate with adjacent osteocytes and cells at the surface of bone through tiny channels known as canaliculi. Osteocytes act as mechanosensors, regulating bone remodelling in response to mechanical stimuli and orchestrating the activity of osteoblasts and osteoclasts to maintain bone health (53).

Last, osteoclasts are large multinucleated cells originating from the monocyte/macrophage lineage of hematopoietic stem cells. The differentiation of these precursor cells into osteoclasts involves: (a) the differentiation of monocytes to macrophages under the influence of the macrophage colony-stimulating factor (M-CSF), and (b) the differentiation of macrophages to osteoclasts under the influence of M-CSF and the receptor for activation of nuclear factor kappa B (NF- κ B) (RANK) ligand (RANKL). Upon activation, the primary function of osteoclasts is to attach to the bone surface, dissolve the hydroxyapatite crystals and degrade the organic matrix, primarily through enzymes like cathepsin K (54).

2.2.4. Bone remodelling

Bone is a dynamic material that undergoes structural changes daily. This involves a synchronised action between the osteoclasts which break down old or damaged bone (bone resorption) and osteoblasts which rebuild it (bone apposition). Apart from these two cell types, osteocytes and mononuclear cells like immune cells play a key role in this process as they orchestrate the migration and differentiation of osteoblasts and osteoclasts by the secretion of cytokines and growth factors. Hadjidakis and Androulakis (7) provide a comprehensive overview of the bone remodelling cycle. It initiates with the migration of partially differentiated mononuclear pre-osteoclasts to the bone surface where they differentiate into osteoclasts and start breaking the old bone matrix, which lasts approximately two weeks. When resorption ends, pre-osteoblasts migrate to the surface where they differentiate into mature osteoblasts that start depositing new matrix which can last up to 4 months. When apposition is completed, lining cells cover the new matrix until the new cycle.

Under homeostatic equilibrium, the amount of bone mass lost during resorption will be replaced with new bone during apposition. In other instances, however, the activity of either the osteoclasts or osteoblasts can prevail leading to bone mass loss or increase, respectively. For example, this is the case for osteoporosis, where bone resorption outpaces bone apposition due to ageing, medical conditions, or unhealthy lifestyle (55).

Essentially, the activity of osteoclasts and osteoblasts depends on the biochemical signals that will guide their differentiation, such as RANKL for osteoclasts and osteoprotegerin for osteoblasts, primarily secreted by osteocytes and have competing effects on each other (53). Based on Wolff's theory (22), key regulating factors for bone remodelling are mechanical stress and strain that bone experiences. Bone deformation forces interstitial fluid through the lacuna-canalliculi network where osteocytes experience shear stress (56). Osteocytes convert this mechanical stimulation to biochemical signals instructing the activity of the surrounding cells involved in the remodelling cycle (57).

Depending on the mechanical stimulation, osteocytes can promote either osteoblastic or osteoclastic differentiation. Intrinsically, osteocytes have a higher capacity to support osteoclastogenesis (i.e., osteoclast differentiation for active bone resorption) compared to osteogenesis (i.e., formation of new bone tissue) (58). Under homeostatic conditions, osteocytes secrete the protein sclerostin (SOST) which inhibits osteoblast differentiation and bone formation by suppressing the Wnt/ β -catenin signalling pathway (57,58). SOST secretion is further elevated during bone disuse benefiting bone resorption (59). In contrast, under mechanical loading, not only SOST levels are downregulated, but additional growth factors (IGF-1), lipids (prostaglandin E₂, PGE₂) and nucleotides (adenosine triphosphate, ATP) that benefit osteoblastic differentiation and bone formation (60).

Previous research has provided estimates for the strain values that bone experiences and determines the process of bone remodelling. Bone resorption tends to dominate at strains below 0.005%, indicating bone disuse and resulting in a reduction in bone mass (61). A homeostatic equilibrium occurs at strains between 0.005% and 0.1%-0.15%, where no significant change in bone mass is observed. At higher strains, bone formation predominates, leading to an increase in bone mass due to the deposition of lamellar bone and minerals (61,62). However, when the strain becomes excessive, woven bone begins to form. Although no definitive threshold for woven bone formation has been established, some studies suggest it occurs at 0.2% (61), while others report thresholds as high as 0.4% (62). These deviations may occur due to load frequency, magnitude, and local bone apparent modulus.

2.2.5. Healing process and osseointegration

Bone healing occurs through two main mechanisms: endochondral ossification (indirect healing) and intramembranous ossification (direct healing). Endochondral ossification typically occurs during normal skeletal development or the union of large fractures in long bones, where a soft cartilaginous callus is initially formed by mesenchymal stem cells at the site of fracture. The callus serves as a scaffold that will gradually be replaced by mature and mineralised bone tissue (63). In contrast, intramembranous ossification involves the differentiation of mesenchymal stem cells into osteoblasts, which deposit new bone matrix without an intermediate cartilage stage. This process typically occurs during the formation of flat bones like the skull or during the healing of small fractures, where the gap between bone surfaces does not exceed 0.01 mm and the interfragmentary movement is low (64).

Osseointegration is a form of intramembranous ossification, which results in the growth of bone tissue directly onto and into the surface of the implant, a procedure that starts three to twelve weeks after implantation and can be continued for up to three years (16,65).

The peri-implant bone healing is initiated by the reaming of the host bone, necessary for the insertion and installation of the implant. The injury caused in the bone results in hematoma and the implant is covered by blood immediately after the installation. This is particularly important as blood contains proteins and platelets which will prepare the implant surface for the next stages as detailed by Kuzyk and Schemitsch (66). Platelets will form a clot which serves as a temporal scaffold to aid the migration of additional cells involved in osseointegration at the implantation site as well as supporting them with

necessary cytokines (such as IL-1, IL-6, IL-8, TNF, and MCS-F), chemoattractants, mitogens, and growth factors (such as TGF- β 1 and 2).

After clotting, the inflammatory response begins with the sequential recruitment of multiple immune cells, as described in (66–68). First, neutrophils arrive at the wound site within the first 30 min to remove debris, bacteria, and necrotic tissue. Monocytes migrate at the site within 24–48 hours, and under the influence of MCS-F and IFN- γ , they are activated and polarise to pro-inflammatory M1 macrophages within 1–2 days. M1 macrophages not only aid neutrophils in clearing the site, but they also release pro-inflammatory cytokines (e.g., IL-1 β and TNF- α) to support the inflammatory response and recruit additional immune cells. After the peak of inflammation, typically around 3–5 days post-injury, anti-inflammatory cytokines like IL-4 and IL-10, produced by the surrounding immune cells, guide the transition of M1 macrophages into M2 macrophages. M2 macrophages are critical for bone healing as they secrete glycoproteins (osteopontin), cytokines (IL-10), and growth factors (TGF- β , BMP-2 and VEGF) which promote cell adhesion, cell proliferation, osteoblastic differentiation, and angiogenesis (67,69). It is important to highlight that at this stage, the timely transition from M1 to M2 is critical for successful tissue regeneration. Prolonged activity of the M1 will cause excessive inflammation and will delay healing, while early M2 polarisation can lead to fibrous tissue formation instead of mature bone tissue (68,70,71).

The completion of the inflammatory phase is followed by the migration of mesenchymal stem cells at the wound site, where they proliferate and colonise the implant surface. The fibrin clot as well as proteins and growth factors previously released at the environment not only aid in the migration of MSCs but also guide their osteoblastic differentiation (16,66). Within the first 24 hours, MSCs will start depositing non-collagenous ECM onto the implant surface which constitutes an afibrillar zone with a thickness between 0.2–0.5 μ m, serving as the cement line (66). As the osteoblastic differentiation progresses, ECM expands and matures, becoming enriched with type I collagen. This unmineralized collagenous matrix is referred to as osteoid. Mature osteoblasts eventually start to mineralise the collagenous matrix, typically with a lag of 2–3 weeks. As the mineralisation progresses, osteoblasts move away from the mineralisation front, however, some become entombed in the newly formed bone matrix and further differentiate into osteocytes (72–74).

This initial matrix is characterized as woven bone, featuring randomly oriented collagen fibrils and irregularly shaped osteocytes, with relatively low mineralization. It serves as a natural scaffold that forms rapidly to fill the gap between the implant and bone within the first four to six weeks (74). Although what was described till now corresponds to the formation of woven bone at the implant surface, an analogous woven bone is formed at the opposite side where the host bone is as an injury response. Interestingly, *in vivo* data suggest that the woven bone formation rate is 30% slower at the side of the host bone, compared to the side of the implant (66). When these two matrices connect and fill the gap between the bone and the implant, they will establish a premature biological fixation. Strong long-lasting biological fixation will be generated when woven bone transforms into mature lamellar bone through the process of remodelling, a process that typically takes 3–6 months.

Last, it is important to mention that the formation of woven bone occurs concurrently with the formation of new micro-vessels. This process is driven by the growth factors (e.g., VEGF) released from macrophages but also from osteoblasts as they differentiate. In this context, successful osseointegration not only encompasses the formation of new bone tissue but also the extension and integration of the blood vessel network within the implant (72,75).

2.2.6. Challenges associated with cementless implants

During joint arthroplasty, surgeons remove the damaged or deteriorated cartilage, exposing the underlying bone. The exposed bone serves as the foundation for the implant, providing the necessary support and fixation. Consequently, the longevity and success of cementless implants depend on preserving bone health through bone remodelling as well as the successful osseointegration.

Data regarding bone modelling makes clear that disruption in physiological bone strain due to the presence of an overly stiff implant will lead to bone resorption and eventually weakening of the bone around the implant (76). Moreover, the narrow strain range for active bone remodelling constitutes implants that match the stiffness of bone essential for eliminating the stress-shielding effect and preserving bone health after surgery (77).

In addition, the multi-facet process of osseointegration demonstrates that the surface of the implant should be tailored to support osteoconduction and vascularisation. This has to be particularly concerning in the case of metallic porous structures, which interests the current work. While metals used in implants are biocompatible (i.e., non-toxic or with minimal inflammatory response), they are not bioactive (i.e., they do not promote cellular attachment, proliferation, and differentiation) compared to bioactive glasses or calcium phosphate ceramics which exhibit a chemical composition similar to that of natural bone (19,78). In addition, angiogenesis and vascularisation of the implant site for oxygen and nutrient supply, as well as space for the removal of waste products, are imperative to avoid cell death or heterogeneous tissue growth. This is an everlasting challenge, especially in large-sized porous structures, where a precocious tissue formation at the outer region of the structure inhibits the oxygen supply towards the core leading to necrosis and lack of vascularisation (79).

Apart from the implant material, an additional challenge during osseointegration is the movement of the implant relative to the bone. Strong osseointegration can take several months and during that time the implant is dependent on mechanical friction to remain stable inside the bone. Minimising implant micromotion is crucial for successful osseointegration. Appropriate values of micromotion can range from tenths to hundreds of micrometres depending on loading time, implant type and material. The limit of tolerable micromotion has been reported in the range of 15 to 750 μm , averaging 112 μm (80). Implants with bone-matching stiffness can yield a more homogeneous stress distribution with physiological stress values compared to stiff implants, and while micromotion around low-stiffness implants may be higher than high-stiffness implants, it remains within a range that enables osseointegration (81).

2.3. Cellular materials and lattice structures

2.3.1. Design, base materials and fabrication methods

Synthetic cellular materials have been made through diverse fabrication methods depending on the base material. Early conventional methods included liquid-state processes (e.g., foaming through gas injection or casting) and solid-state processes (e.g., powder sintering using space holders or hollow microspheres) which generate foams with open or closed interconnected pores made of metals, polymers, ceramics, and ceramic-polymer composites (82–84). Tuning of the respective process parameters can result in foams with controlled porosity and pore size/shape (39,85). Nevertheless, all the above methods result in randomly distributed pores which greatly compromises the mechanical properties of foams. More importantly, while inexpensive in manufacturing, foams lack reproducibility

which hinders their establishment in most industrial applications due to inconsistency in morphology and properties (86).

As a solid alternative, bottom-up manufacturing methods, such as additive manufacturing (for metals, polymers, and ceramics) and electrospinning (only for polymers), typically allow for more precise control over the structure morphology and enable the fabrication of structures with wide scalability and high complexity. In the context of additive manufacturing, the synthetic cellular materials are termed lattice structures and constitute 3D networks of struts (i.e., beams) or sheets (i.e., plates) which can form periodic or stochastic porous structures.

Periodic lattice structures consist of geometrically defined and spatially repeating unit cells (86). In the case of strut-based lattices, unit cells are typically designed to represent crystal structures (e.g., the body-centred cubic and face-centred cubic), Platonic and Archimedean polyhedra (87), with some widely used shown in **Figure 2.2**. In the case of sheet-based lattices, unit cells are designed based on triply periodic minimal surfaces (TPMS), such as the Schoen gyroid and Schwartz diamond, which are smooth and infinitely repeating surfaces creating a boundary between solid and void sections of the structure (86,88).

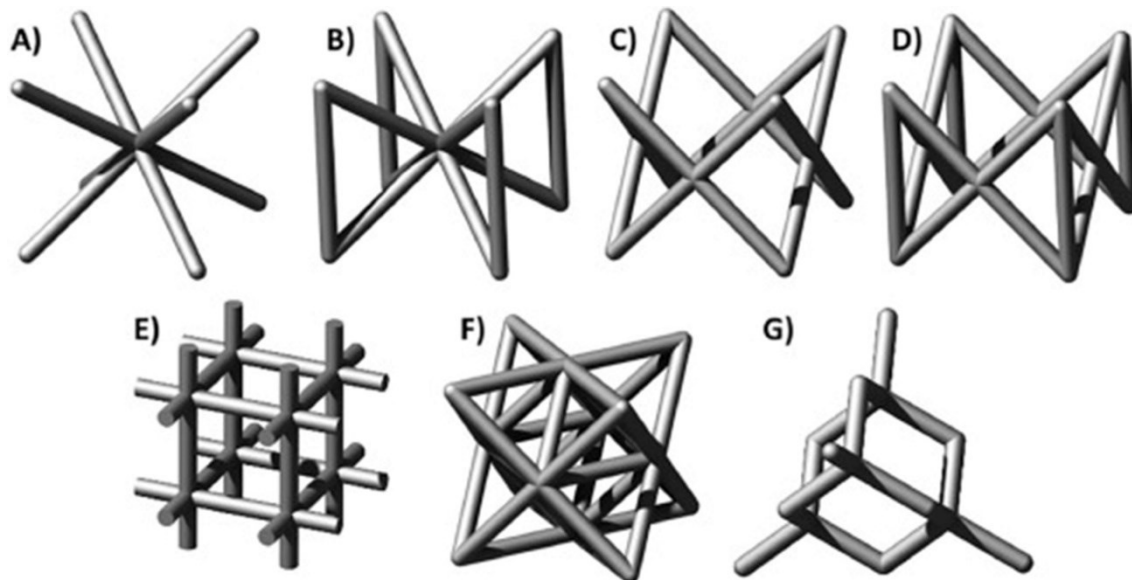


Figure 2.2. Examples of widely used periodic strut-based unit cells. (A) BCC, (B) BCC-Z, (C) FCC, (D) FCC-Z, (F) Cubic, (G) Octet-truss, and (H) diamond. Figure reproduced under CC BY license from (86).

Stochastic lattice structures can be both strut-based and sheet-based. Strut-based stochastic structures are typically generated by connecting randomly distributed points derived from statistical distributions (e.g., Gaussian and Poisson) or the edges of the Voronoi diagrams (89–91). Similarly, sheet-based stochastic structures can be generated by segmenting a 3D volume into randomly shaped subregions which are then represented with randomly oriented periodic TPMS (92) or randomly defined surfaces (93). These methods enable the creation of pseudo-random structures with often controllable micro-architectures. Random points are generated using seed numbers which enables the reproduction of the same random lattice at any time. This capability is a significant advantage over early forms of synthetic cellular materials, like foams, where micro-architecture was often difficult to control and reproduce.

Each lattice type incorporates distinct advantages and disadvantages, thus the selection of one should depend on the application.

Periodic versus Stochastic

Periodic structures offer more predictable mechanical behaviour owing to their regular and repetitive micro-architecture. In addition, they have been generally shown to exhibit superior mechanical properties than stochastic lattices for both strut-based (94) and sheet-based (92) structures. This is often attributed to the randomness of stochastic structures which can introduce weak (less supported) regions inside the specimen (95). However, as discussed next, mechanical behaviour greatly depends on several factors (e.g., porosity and topological characteristics) making a fair comparison between periodic and stochastic lattices challenging. From a design point of view though, stochastic designs benefit the fabrication of lattice-based parts with irregular or curved geometry. While stochastic structures can easily represent such geometries by relying on randomly populated points, periodic structures rely on computational algorithms to change the size/shape of their unit cells and conform them to the part's external surface (96,97).

Strut-based versus sheet-based

Sheet-based lattices are often recognized for their superior mechanical properties, which are attributed to their higher surface area-to-volume ratio and smoother surface transitions (98–101). The continuous nature of these surfaces leads to more uniform stress distribution, reducing the likelihood of localised stress concentrations that can occur in strut-based lattices. In contrast, the discrete nature of strut-based lattices can result in stress concentrations at strut junctions, making these structures more sensitive to local defects and introducing anisotropic behaviour. However, strut-based lattices are generally easier to design and more straightforward to manufacture, especially in the case of additive manufacturing. Due to their complex geometries and large surface areas, sheet-based lattices often require additional support structures to manage overhanging surfaces and prevent warping during fabrication. Furthermore, the large surface area in sheet-based lattices can lead to higher entrapped porosity and potential defects due to excessive local energy deposition during additive manufacturing (99).

2.3.2. Mechanical behaviour under quasi-static loading

The mechanical performance of synthetic cellular materials primarily depends on three factors: (a) the base/parent material, (b) the relative density (or porosity), and (c) the topology (or micro-architecture).

Base material

The base material directly impacts the overall behaviour of the structure as it dictates the material's intrinsic properties, such as strength, stiffness, ductility, and toughness. For instance, metals like titanium or aluminium, known for their high strength-to-weight ratios, allow the development of lightweight structures with excellent load-bearing capabilities, making them ideal for aerospace and automotive applications (86). On the other hand, polymers with high flexibility and low density, are well-suited for foams designed for energy absorption and impact resistance, as they can undergo large strain deformation (50%-60%) under low stress without catastrophic failure (102).

The ductile or brittle nature of base material is particularly critical in determining how a porous structure responds to mechanical loads (95). Ductile materials like polymers and most metals, allow structures to deform plastically under load with their struts/walls undergoing extended bending or buckling. In contrast, porous structures from brittle materials like ceramics although exhibit high stiffness and strength, they are more prone to catastrophic failure without significant plastic deformation due to sudden fracture of their struts/walls. These differences are also apparent for the same material type, for

example, the single-phase commercial pure titanium (CP-Ti) is known to be more ductile than the alloyed Ti-6Al-4V, making it significantly weaker but capable of undergoing higher plastic deformation without fracture (103).

Relative density

The porosity of a structure, meaning the aggregate volume of voids inside the structure divided by the structure's volume is directly correlated to relative density (the density ρ of the porous structure divided by the density ρ_s of the parent material):

$$\text{Porosity} = 1 - \frac{\rho}{\rho_s} \quad \text{Equation 2.1}$$

Relative density (or porosity equivalently) is a central factor for the mechanical behaviour of a porous structure and is directly related to the size of the pores. Increasing pore size results in decreased relative density and a generic approach for increasing pore size is by increasing the edge length L of the unit cells that form a structure or by decreasing the strut/wall thickness t (104). Analytical expressions correlating relative density with the two structural features (t and L) that form the pores can be derived from the unit cell geometry in regular porous structures (105). For generalisation, Gibson and Ashby (106) proposed the below approximate expression for the relative density of open porous structures:

$$\frac{\rho}{\rho_s} \propto \left(\frac{t}{L}\right)^2 \quad \text{Equation 2.2}$$

Although this simplified model considers any porous structure as a cubic array with unit cell size L and wall thickness t (**Figure 2.3A**), it has been proven adequately accurate for both periodic and stochastic porous structures of various geometries.

For relative densities higher than 20% a higher order is considered more accurate to predict relative density:

$$\frac{\rho}{\rho_s} = C \left(\frac{t}{L}\right)^2 + D \left(\frac{t}{L}\right)^3 \quad \text{Equation 2.3}$$

Where C and D are constants of proportionality calculated using experimental data.

Topology

Topology refers to the connectivity and spatial arrangement of struts, and the shape of the unit cell edges (95). It is a major design parameter that dictates how external loads are distributed within the structure, and how unit cells deform (**Figure 2.3B**) and the whole structure deforms under loading.

Particularly nodal connectivity Z (the number of struts connected to each node) is considered decisive for the micro-deformation of the structure's elements (struts or walls) which can undergo bending, stretching or even buckling. Under quasi-static loading, this is typically explained through the Maxwell criterion for beam frames (95). Based on the number of struts s and nodes j , the Maxwell number M for 3D frames is calculated as:

$$M = s - 3j + 6 \quad \text{Equation 2.4}$$

For a high connectivity structure $M \geq 0$, struts experience axial loads (stretch-dominated deformation) and the structure appears rigid as a statically indeterminate frame. While for a low connectivity structure $M < 0$, struts exhibit bend-dominated deformation and the structure appears compliant as a pin-jointed mechanism.

A third category, buckling-dominated structures, involves elements that exhibit buckling and a sudden loss of load-carrying capacity at a critical stress level, leading to a nonlinear mechanical response. This behaviour is not linked to the Maxwell criterion and connectivity. Rather it is primarily driven by relative density and the arrangement of struts. For instance, rigid polymer and metallic foams buckle when relative density is $\leq 5\%$ and $\leq 1\%$, respectively (95). This is attributed to the t/L ratio, also known as the slenderness ratio, which informs the propensity of a structural element to buckle, and its threshold value depends on the base material (107). Alternatively, buckling can occur to unit cell topologies whose structural elements exhibit high alignment with the loading direction. For example, this is the case for the cubic unit cell (108) when loaded normally, where half struts are parallel to the load and the other half are vertical to the load.

Bend-dominated and stretch-dominated micro-deformation modes

Under quasi-static compression, the stress-strain curves of cellular materials include three regions that appear sequentially: (a) the elastic/linear region, (b) the plastic deformation, and (c) the densification. The plastic region starts after a first peak load (typically termed as ultimate compressive strength) has reached and is the part of the stress-strain curve that differs among the major strut micro-deformation modes. The form of the stress-strain informs about the macroscopic response and failure mechanism of the whole structure.

A bend-dominated structure (**Figure 2.3B**) exhibits an elastoplastic-like stress-strain model with the material being plastically deformed under an almost constant stress which is approximately equal to the compressive strength. This occurs due to the low connectivity of the structures that allow struts to deform freely through bending. This constant stress is called plateau σ_{pl} and continues until the structure's densification begins (typically occurs between 30% to 50% strain).

In contrast, the tightly interconnected struts in a stretch-dominated structure cannot bend freely and experience axial stretching/compression to resist external compression load. After a peak load is reached, individual struts fracture (typically generating a shear/crush band) which results in a sudden drop of stress due to structural relaxation, referred to as post-yield softening (2) (**Figure 2.3C**). Following the softening, stress will either consolidate at a constant plateau value (meaning that structure fails by broadening the developed crush band) or will fluctuate due to the fracture of additional struts (generation of multiple crush bands). Extended plastic deformation will again lead to the point of densification.

This last region is common to all micro-deformation modes; the opposite sides of cell walls start to impinge causing stress to rise steeply.

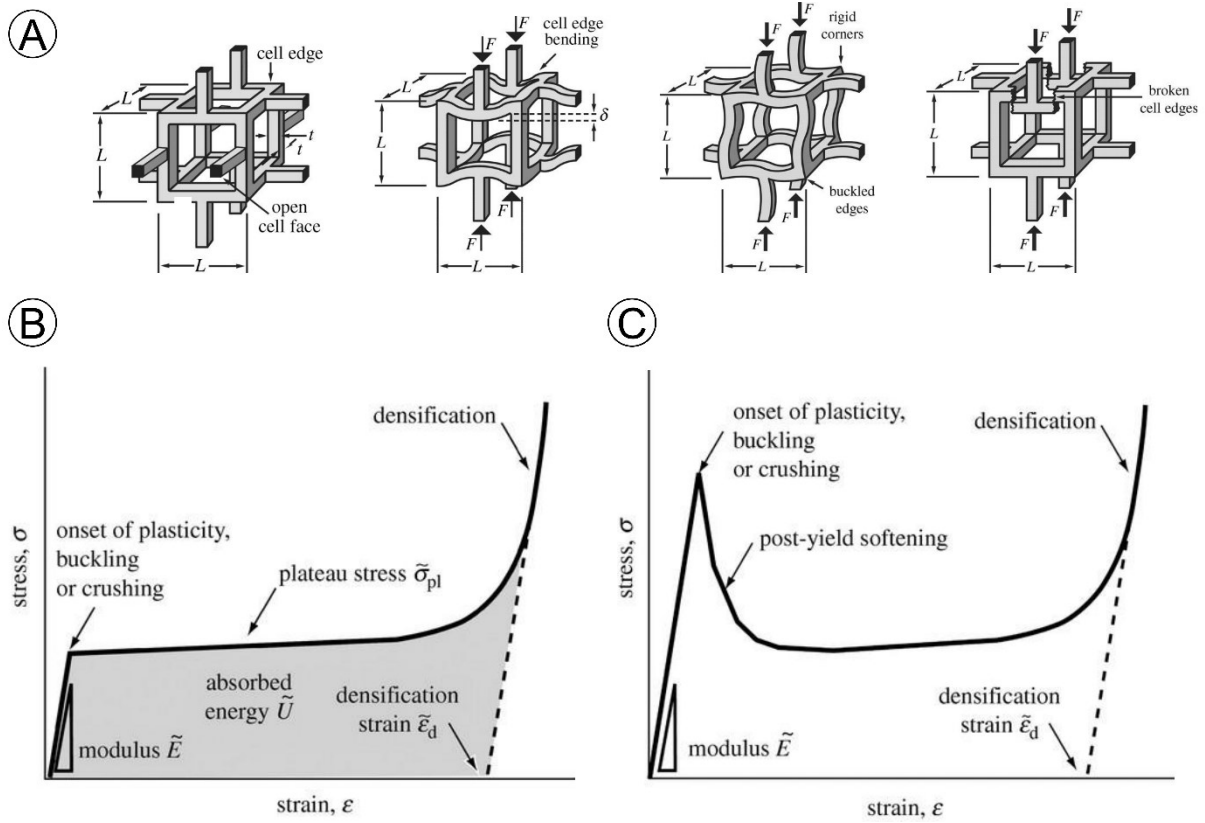


Figure 2.3. Different micro-deformation modes under quasi-static compression. (A) The Gibson-Ashby model for materials with open pores, and major failure mechanisms of cell struts through bending, buckling and fracture. Different macroscopic stress-strain curves are generated in structures with (B) bend-dominated and (C) stretch-dominated micro-deformation modes. Figures reproduced from (95) with permission from ROYAL SOCIETY (License No. 1581193-1).

Using the same analogue model, Gibson and Ashby proposed that the two major mechanical properties of a porous structure: the compressive strength σ_u ¹ and the elastic modulus E , are correlated to relative density via a power law as shown below:

$$\frac{\sigma_y}{\sigma_{ys}} \propto \left(\frac{\rho}{\rho_s} \right)^{n_1} \quad \text{Equation 2.5}$$

$$\frac{E}{E_s} \propto \left(\frac{\rho}{\rho_s} \right)^{n_2} \quad \text{Equation 2.6}$$

The values of power indices n_1 and n_2 depend on the deformation mode. Based on the theoretical model, n_1 should be equal to 1.5 and 1 for bending-dominated and stretching-dominated structures, respectively. While n_2 should be equal to 2 and 1 for bending-dominated and stretching-dominated structures, respectively. The values of power indices make apparent that structures with low nodal connectivity exhibit lower mechanical properties than structures of high connectivity of the same relative density (95,109). Previously published results have shown that indices values might lay between, below or above these theoretical values— typically between 1 to 3 for the majority of experimental data (86).

¹ Some authors refer to the peak load as “yield”. While this term is valid as it indicates the end of structure’s elastic region, international mechanical testing standards of solid and cellular materials define yield strength as the 0.2% linear offset at the elastic region of the curve. This work adapts this terminology and distinguishes the yield from the ultimate compressive strength.

Yet, this set of equations constitutes a useful tool for choosing porous structure topologies. This also explains the reason that bend-dominated structures are typically used for energy absorption applications where a plateau region is necessary, while stretch-dominated structures are typically used in load-bearing applications where a high strength-to-weight ratio is required.

However, when designing a bone replacement structure, maximising strength in terms of stiffness (elastic modulus) is more critical. By combining these two equations, a modified expression of the Gibson-Ashby model can be obtained (48):

$$\frac{\sigma_u}{\sigma_{ys}} \propto \left(\frac{E}{E_s} \right)^{\frac{n_1}{n_2}} \quad \text{Equation 2.7}$$

The strut micro-deformation modes have been extensively studied for various periodic unit cell topologies using experimental, computational, and analytical approaches (110,111). Using analytical calculation, a nodal connectivity of at least 12 has been shown to be necessary for 3D periodic structures to be statically indeterminate and stretch-dominated (112). A similar approach is not feasible for stochastic structures, and less data exist showing how the topological characteristics may influence the micro-deformation mode within stochastic structures. Early studies on foams have shown that they exhibit bending-dominated behaviour, as weak areas in their heterogeneous pore network tend to downgrade the mechanical properties of the whole structure (95). For more controlled stochastic lattice structures, Mueller et al (91) carried out a computational analysis with stochastic lattices employing connectivity from 4 to 14 but did not find a transition from a bending-dominated to a stretching-dominated behaviour with increasing connectivity. In experimental work, Raghavendra et al (94) considered connectivity levels between 4 and 6 and found stochastic structures had inferior quasi-static and fatigue properties compared to periodic lattices, which was attributed to the bending-dominated behaviour of the stochastic structures. This highlights the necessity for more experimental data to study stochastic structures in depth.

Limitations of the Gibson-Ashby model

The model utilises normalised values of the mechanical properties, making it seem independent of the base material. Hedayati et al (113) justified that both topology and base material have independent effects on the mechanical properties of lattices. By comparing lattices made with three different unit cells (truncated cuboctahedron, diamond, and rhombic dodecahedron) and three different materials (CP-Ti, Ti-6Al-4V, CoCr), they found that different topological designs and material types can introduce up to “10-fold and 2-fold difference” in the normalised compression properties, respectively. Using a fixed stochastic lattice design made from CP-Ti, Ti-6Al-4V, Ta and Ti-Ta (mixture of CP-Ti and Ta powders), Ghouse et al (114) showed that material type affects both the shape of the curves and the resulting Gibson-Ashby regression models. Notably, while having the same lattice topology, the brittle Ti-6Al-4V specimens were the only ones exhibiting strong stretching-dominated characteristics (high stiffness and strength, and apparent post-yield softening). Similar observations about the effect of base material on the stress-strain curve morphology were reported for a stochastic design made with the Ti-6Al-4V and the more ductile stainless steel 316L (89) and a periodic design (with the dodecahedron unit cell) made with Ti-6Al-4V and CP-Ti (103).

The above results highlight the critical effect of base material which can make a theoretically bend-dominated lattice exhibit stretch-dominated characteristics. This also suggests that independent models should be developed to predict the mechanical performance of lattices for different topologies and base materials.

Structural anisotropy

Cellular materials and lattice structures can exhibit high anisotropy in their mechanical properties due to their inherently directional architecture. In directions where struts are aligned with the load, the structure typically shows higher stiffness and strength while in directions where the load is applied transversely to the struts, the mechanical properties can be considerably lower (115).

Differences in the mechanical response based on load direction were first perceived in specimen cores removed from cortical (116) and trabecular bone (117) which is attributed to the alignment of lamellae and trabeculae, respectively. Structural anisotropy in bone samples is primarily quantified using high-resolution μ CT scans which allows the visualisation of its structural elements. Using the spatial orientation of the elements, a second-order fabric tensor can be generated using computational methods such as the mean intercept length, the volume orientation distribution, and the star volume distribution. The tensor can be further visualised as an ellipsoid with three principal axes (derived from fabric eigenvalues) (118). The fabric eigenvalues are used for calculating the degree of anisotropy (ratio of highest to lowest eigenvalues) (119) and the shape of the ellipsoid indicates any preferential orientation of the structure: a more elongated ellipsoid means a highly oriented structure (high degree of anisotropy), while a more spherical ellipsoid means a more isotropic structure (low degree of anisotropy) (120). This method can be extended to artificial lattice structures using either μ CT data (121) or by calculating the orientation of the structural elements directly from the CAD models (122).

Synthetic structures can exhibit directional deviation in the measured mechanical properties due to structural anisotropy (related to topology) and structural defects (related to the manufacturing process). Anisotropy in foams, for instance, originates from the heterogeneity of pore shapes and defects, such as buckled or broken struts and results in differences of up to 30% between moduli in different directions (83). A representative example from periodic lattices is the highly interconnected octet-truss lattice which has been shown to exhibit $\approx 40\%$ lower stiffness when loaded normally to the unit cell's face than when it is loaded along the unit cell's spatial diagonal (123).

Apart from the property values, orientation has been shown to affect the failure mechanism in various lattice topologies such as the cubic, BCC and cross (124,125). Non-equivalent deformation of pores across different directions can lead to structures having more brittle characteristics, typically associated with stretch-dominated behaviour, contrary to what Maxwell's criterion would suggest (126).

These observations have led researchers to manipulate lattice topologies to enhance structures' strength and stiffness along distinct directions. In one example, the BCC unit cell can be designed with vertical struts connected to the central node (also called BCC-Z unit cell) (127). In another one, the FCC lattice was designed by rotating its unit cells to induce different failure mechanisms (128).

The aspect ratio of the unit cells is another factor that has been shown to greatly affect the mechanical properties and deformation depending on the load direction in both (129) and periodic lattices (127,130). Considering a Cartesian coordinate system, more elongated unit cells (or pores) along the Z-axis result in higher compressive strength and higher elastic modulus along Z than X and Y, and a lower Poisson ratio in the XZ and YZ planes.

The above indicates that structural anisotropy can significantly impact lattice mechanical performance. In most cases, uniaxial compression tests are inadequate to fully characterise a lattice behaviour in real applications where loading occurs in multiple directions. Ultimately, the use of lattice structures with highly isotropic properties would benefit the accurate prediction of lattice-based components *in situ*. While this is typically difficult to achieve with periodic structures due to the repeated struts orientated

to distinct angles, evidence suggests that can be feasible with stochastic structures that employ struts along all directions (115).

Size and edge effects

Size and edge effects refer to the phenomena where the mechanical properties of cellular materials are influenced by their dimensions and boundaries. Smaller structures may exhibit different strength, stiffness, and failure mechanisms compared to larger ones due to the influence of increasing surface-to-volume ratio and the amplified influence of structural defects. Edge effects arise from the differences in the mechanical environment experienced by cells at or near the boundaries of the material compared to those in the interior.

Size and edge effects usually act synergistically in small-sized specimens as damaged or free struts at the edges of the small specimens do not contribute to strength by carrying load while they increase the apparent cross-sectional area or moment of inertia of testing specimens (131,132). This coupled effect is primarily apparent in metallic foams which tend to exhibit lower stiffness, compressive strength and bending strength when specimens are critically small. However, macroscopic properties eventually converge at constant values when the structure is formed by a large enough number of unit cells (133). Early studies have demonstrated that convergence occurs when the shortest edge of the specimen is composed of at least 5-8 unit cells (134,135), and the converged properties are referred to as “bulk” material properties.

There are, however, cases where small specimens may exhibit higher mechanical properties than large specimens. This is often linked to the brittle nature of the base material and the increasing number of structural defects found in larger specimens (Weibull theory). More defects and imperfections may lead to randomised fracture of struts at distant regions in larger specimens that will compromise the strength and stiffness compared to small specimens where defects are statistically fewer (95,135).

International testing standards for measuring lattice properties (such as the ISO 13314 and ASTM D1621) are based on the idea that mechanical testing of a small specimen of a lattice is representative of the structure when deployed in an application. The standards require specimens to be adequately large (consisting of at least 10 unit cells across the width to avoid size effects) with an aspect ratio (specimen height to diameter/width ratio, $\zeta = H/D$ or H/W) between 1 and 2 (136,137).

Specimen's aspect ratio is another measure that in extreme values is known to affect mechanical properties under compression. In the case of solid materials, large aspect ratios lead to buckling while too small aspect ratios can induce end effects where the specimen deforms to a barrel shape owing to high friction at its interface with the platens. Neither of these is indicative of the compressive behaviour of the structure and should be avoided. A maximum aspect ratio equal to 3 is considered proper to avoid buckling for ductile materials, and 1.5 to 2 for brittle materials, while sufficient platens' lubrication can eliminate edge effects for low aspect ratios (138).

The effect of aspect ratio in cellular materials has primarily been investigated for the uniaxial compression of trabecular bone cores. Linde et al (139) showed that increasing the aspect ratio of the test specimen increased the measured stiffness. The same trend for stiffness was found by Ang et al (140) who further showed that increasing the aspect ratio of the test specimen reduced the measured ultimate strength. These studies, however, employed specimens with relatively small sizes making them prone to size effects due to structural artefacts (141).

Limited data exist for synthetic porous structures and conclusions are contradictory. In sandwich panel structures, increasing the aspect ratio was reported to reduce the measured modulus and strength (142). However, in polymeric foams, increasing the aspect ratio was reported to increase the measured

modulus but conversely decrease the measured strength (143). In magnesium foams, increasing the aspect ratio was also reported to reduce the strength (144). Other works in periodic lattices demonstrate either increasing (145) or decreasing (146) modulus for increasing aspect ratio. Still, specimens in these works consisted of few layers and cells, hence it was not clear whether the stiffening effect originated from the macroscopic aspect ratio or edge/size effects (147,148). Interesting findings from numerical studies of periodic lattices report that altering the aspect ratio results in different strain distributions inside the structures (149–151) suggesting it may affect the measured properties of the structure.

2.3.3. Mechanical behaviour under cyclic loading

Uniaxial fatigue tests are carried out on tension-tension, tension-compression and compression-compression conditions using sinusoidal waveform loading which is controlled through frequency f and stress ratio R until catastrophic failure or a threshold number of cycles is reached. The stress ratio is calculated using the absolute values of the applied stresses:

$$R = \frac{\sigma_{min}}{\sigma_{max}} \quad \text{Equation 2.8}$$

In this relation, σ_{max} is considered the testing stress level and represents the fatigue strength $\sigma_{f,N}$ – the maximum stress that can be applied for the material to survive N cycles for fixed f and R . Further, by testing a series of stress levels, a logarithmic curve connecting all stress level and cycle data points can be generated. This is known as the S-N (or Wohler) curves and are used to predict the fatigue life of a mechanical component or material under a certain applied stress. Increasing R has been shown to shift the S-N curves upwards (longer fatigue life) both in metallic foams (152) and AM lattice structures (153).

Based on cycle number, the S-N curves can be divided into three main regions:

- The low-cycle fatigue finite life (typically $N < 10^3$)
- The high-cycle fatigue finite life (typically $10^3 < N < 10^6$)
- The endurance limit (typically $N > 10^6$)

Low-cycle fatigue typically involves large plastic deformations, while high-cycle fatigue is characterised by relatively small deformations with minimal plasticity (138). In the last region, the S-N curve exhibits a sudden curvature change (knee point) and becomes horizontal. The stress level at which the curve is horizontal represents the endurance limit below which the material can practically survive infinite loading cycles. In the case of solid metals, the endurance limit is typically estimated at 10^7 cycles. While solid metals can be tested at high frequencies (> 100 Hz), cellular materials should be tested at lower frequencies (typically 15 Hz) primarily to avoid significant heating due to internal friction and hysteresis losses but also to better replicate the physiological rate of loading – e.g., 1 to 90 Hz for knee (154,155). The use of low frequencies though makes high-cycle fatigue testing ($> 10^6$ cycles) excessively time-consuming. This has led researchers to test the fatigue strength of cellular materials up to 10^6 cycles as a representative measure of structures' longevity. Nevertheless, some studies have shown no effect of high frequency on the measured mechanical properties. No change in the fatigue life of closed-cell aluminium foams was seen when tested at frequencies between 1 and 70 Hz in (156), but also in the fatigue life of open-cell titanium lattices when tested at 15 and 110 Hz in (157).

Similarly to quasi-static conditions, the fatigue strength of cellular materials and lattice structures depends on the base material, relative density, and topology. Hence, to allow direct comparison of intrinsically different structures, fatigue strength $\sigma_{f,N}$ is usually presented normalised to the yield or ultimate strength of the structure under quasi-static conditions.

Fatigue mechanisms

During cyclic compression, the material's stiffness and strength drop with increasing number of cycles owing to plastic deformation of pores and cracking of struts. Ashby et al (83) highlight how different loading modes influence the fatigue failure mechanism on aluminium foams. Under tension-tension, foam elongates plastically due to cyclic ratcheting (cyclic creep) until a macroscopic fatigue crack develops (around 0.5% plastic strain) and starts to progress towards the weakest sections of the structure until final failure (brittle fracture). While, under compression-compression material exhibits a ductile behaviour: an initial increase in strain (stage 1) is followed by a plateau region where cracks initiate but plastic strain remains constant over the increasing cycles (stage 2, also termed as incubation period). While cycles progress, large plastic strains accumulate due to strut cracking and cyclic ratcheting propagation until the final fracture occurs. This is stage 3 and can be manifested in three ways:

- Uniform accumulation of plastic strain across the specimen without the formation of a crush band (Type I).
- Generation of discrete crush bands at random weak regions of the specimen (Type II).
- Generation of a single crush band which propagates and broadens uniformly over cycles (Type III).

The different fracture modes at stage 3 are also apparent through the strain-cycles plots (**Figure 2.4**): a straight vertical line indicates uniform strain accumulation during type I or Type III modes, in contrast, a fluctuating strain increase (involving numerous "jumps") corresponds to a type II mode.

A failure criterion is needed to indicate the end of the fatigue test. Material separation is an obvious and appropriate criterion for tension-tension loading, while different distinct points from the strain-cycles curve are picked in the case of compression-compression loading. One option is to identify a "knee" on the curve, which indicates the start of Stage 3. This typically occurs at a compressive strain of 1% to 2%, and the associated number of cycles is taken as the fatigue life. Alternatively, as a more straightforward method and less sensitive to graph morphology at stage 3 (158), a threshold stain value is taken as the failure point, e.g., 5% (159), 10% (160), and 20% (161).

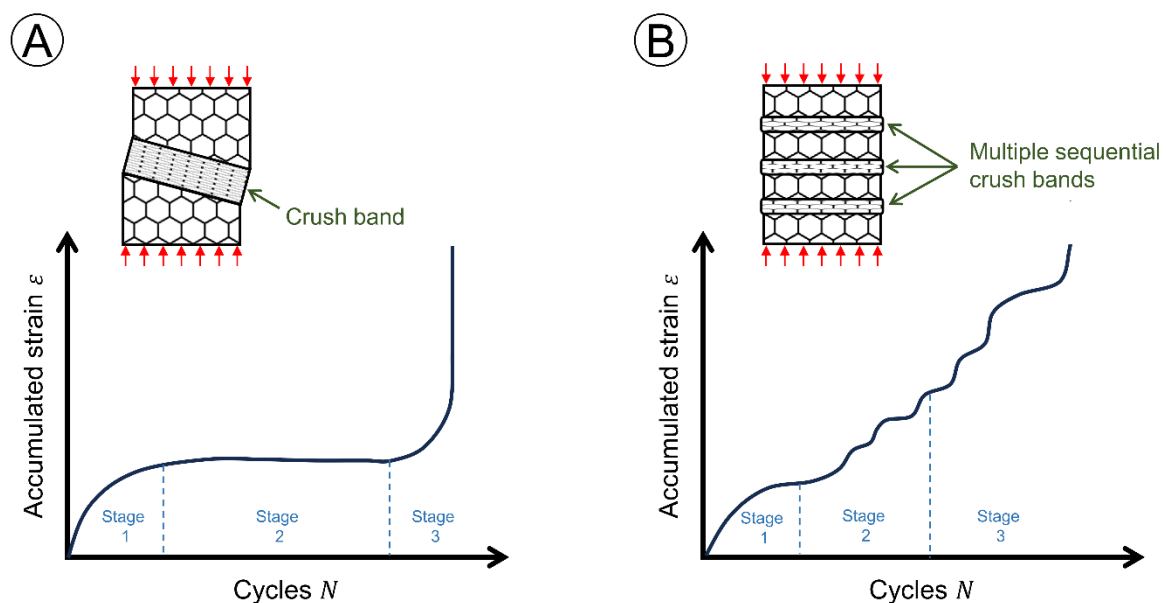


Figure 2.4. Different types of failure mechanisms under cyclic compression. Examples of strain-cycle plots for specimens failing through (A) a distinct crush band and (B) multiple independent crush bands.

The effect of base material and manufacturing process

Yield strength, ductility, crystal microstructure, the presence of multiple material phases, the size and orientation of grains, and the presence of structural defects are widely known to affect the fatigue performance of solid materials (138).

Yield strength and ductility are usually inversely related, most metals present a ductile nature which allows them to undergo high plastic strain without failure, while ceramics and glasses are typical examples of brittle materials that exhibit high yield strength with low plastic deformation capacity. Ductile materials undergo progressive accumulation of plastic deformation, which generates multiple slip bands as cycles are progressing. While the generation of slip bands may reach a saturation point, some of these bands can evolve into cracks. These cracks propagate through the material and eventually merge into a single large crack, causing material failure (138,162). On the other hand, brittle materials are known to be sensitive to structural defects/flaws such as inclusions, grain boundaries, and pores, which act as crack initiation sites. These cracks propagate rapidly due to the material's low plastic deformation capacity, eventually meeting each other and leading to a single catastrophic crack (138).

Ductile materials generally have superior fatigue life under high strain amplitudes because of their ability to undergo plastic deformation before crack propagation. In contrast, brittle materials may perform better under low applied strains due to their high yield strength (163). Nevertheless, the sensitivity of brittle materials to defects limits their extended application in cases that involve cyclic or multiaxial loading where durability and resistance to crack growth are critical.

Crack propagation can be impeded when meeting inclusions, secondary phases, and grain boundaries, making the size and orientation of microstructure grains critical for fatigue strength (164). Smaller grain sizes introduce more boundaries that prevent the movement of dislocations within the microstructure, leading to higher crack initiation and growth resistance (Hall–Petch relationship). Ultrafine microstructures (<10 nm), however, will eventually lead to an inverse phenomenon where grain boundaries will slide unresistingly. Depending on the loading direction relative to the grain structure, materials with elongated or textured grains may exhibit different fatigue strengths. Grains misaligned with loading direction facilitate crack initiation and transgranular propagation due to the development of high shear stresses as explained through Schmid's law (165,166). Still, grain size stands out as the most critical factor for fatigue performance compared to orientation (167).

The number of phases direct the type of microstructure which in turn greatly influences fatigue performance. Major types are the equiaxed (typically formed in single-phase materials or after complete recrystallization), the lamellar (two-phased where the phases alternate in a layered arrangement), duplex (a mixture of equiaxed grains and lamellar colonies), and the Widmanstätten (two-phased where one phase appears as needle-like precipitates, typically formed owing to rapid solidification) (168). The influence of each one in fatigue strength has been investigated thoroughly for both solid and porous materials (168,169). As a representative example for lattice structures, Ghose et al (114) compared the fatigue performance of the same lattice made of CP-Ti, Ti-6Al-4V, Ta and Ti-Ta. They found that Ta and Ti-Ta specimens exhibited the highest fatigue strength at 10^6 cycles over fixed relative density or modulus which was attributed to better surface quality, higher ductility, and favourable microstructure. Regarding the microstructure, the Ti-Ta was found to exhibit a martensite $\alpha'' + \beta$ which is known to exhibit higher fatigue resistance and decreased notch sensitivity compared to CP-Ti's α phase or Ti-6Al-4V's acicular $\alpha' + \beta$ phase (170). Similarly, by comparing the same lattice made of CP-Ti and Ti-6Al-4V, Wauthle et al. (103) found that the ductile CP-Ti had better fatigue strength in high-cycle loading ($>10^5$ cycles), while the brittle Ti-6Al-4V had better performance in low-cycle ($<10^5$ cycles). These results suggest that a brittle metal with high yield strength may perform better at low-cycle fatigue. Still, in the

case of high-cycle fatigue applications where stress is considerably low, and the yielding effects do not dominate the behaviour, ductile metals are favourable. In addition, as discussed later, it underlines the importance of applying heat treatment post-processing to achieve a more favourable microstructure.

The effect of relative density

In analogy to quasi-static strength, higher relative density (i.e., lower porosity) leads to higher (absolute) fatigue strength in porous materials (171). Due to the apparent connection with static strength, looking at the fatigue strength normalised to static strength or modulus can better isolate the true effect of relative density.

Hrabe et al (158) produced Ti-6Al-4V structures with the diamond unit cell, constant strut thickness of $\approx 400\text{ }\mu\text{m}$ and relative densities ranging from 17% to 40% by varying the pore size (500-1500 μm). After normalising to yield, the S-N curves showed that structures with higher relative density had also higher fatigue life. The normalised fatigue strength at 10^6 cycles of the lattices ranged from 0.15 to 0.25, while the normalised strength for a solid specimen with the same Ti microstructure was 0.4. Yavari et al (172) produced Ti-6Al-4V structures with the dodecahedron unit cell and relative densities ranging from 16% to 32% by altering strut thicknesses (140-250 μm) and pore sizes (500-600 μm). As expected, both the absolute and the yield-normalised S-N curves were more elevated for higher relative density indicating higher fatigue life. It is worth mentioning, that in both studies specimens fractured similarly regardless of relative density.

The effect of topology

Different topologies have also been found to affect fatigue life and strength of lattice materials.

Zhao et al (173) compared the fatigue behaviour of Ti-6Al-4V lattices made with similar relative density, but different unit cells: the buckling-dominated cubic, the bend-dominated G7 and rhombic dodecahedron. Results suggested that the buckling-dominated cubic lattice exhibited a lower rate of cyclic ratcheting ($d\epsilon/dN$) and increased fatigue strength (normalised to static plateau stress) than the bending-dominated lattices. This finding was attributed to different internal stresses developed in each case, which are entirely compressive during buckling-dominated deformation and a mix of compressive and tensile during bending-dominated, making the latter more prone to fatigue crack propagation. In line with these results, Peng et al (174) compared lattices of different unit cells (cubic, BCC, FCC) and relative densities using a computational model and found that the cubic lattice had the highest fatigue strength – both absolute and normalised – while the BCC lattice had the lowest.

In (175), CP-Ti lattices with different unit cells (tetrahedron and octahedron) and pore sizes (500 μm and 1000 μm) were tested. As expected, the lattices with the higher connectivity octahedron cell were found to have higher static and fatigue strength than their tetrahedron counterparts for the same pore size.

In an extensive study, Ahmadi et al (160) compared lattices built with different materials (CoCr, Ta, Ti64 and CP-Ti), unit cells (truncated cuboctahedron, rhombic dodecahedron, and diamond) and relative densities (ranging from 0.21 to 0.42). When isolating the effect of material, CoCr had the highest normalised fatigue strength followed by CP-Ti, Ta and Ti-6Al-4V. However, each material was found to have different sensitivity when altering the unit cell and relative density. Namely, normalised S-N curves for different relative densities of CoCr specimens had no significant difference when made from the diamond unit cell, while showed significant differences when made from the truncated cuboctahedron and the rhombic dodecahedron unit cells. The sensitivity in relative density and topology was lower for the other materials. This was attributed to the post-yielding hardening of bulk CoCr which results in normalised fatigue strength higher than 1, with the authors concluding that “as the relative density

increases, the fatigue response of porous CoCr structure becomes more similar to the bulk CoCr material and topology contributes less to the fatigue response”.

Another example of topology investigation comes from Speirs et al (176) who compared NiTi lattices with the octahedron unit cell and the TMPS gyroid in a cellular and a sheet configuration. All specimens failed with a diagonal shear band formation, but the absolute and normalised fatigue life of sheet and cellular gyroid were significantly higher than the octahedron. The superiority of sheet-based compared to strut-based lattices was also shown in (177) where the sheet-based gyroid was compared to the strut-based FBCCZ (combination of FCC and BCC-Z). Both types exhibited the same relative density and were tested under cyclic tension-compression loading. The study found that the gyroid lattice demonstrated better fatigue life with S-N curves approximating the ones of the solid base material (stainless steel 316L). Both studies concluded that the better cyclic performance of gyroid lattices originates from the lower stress concentration in the nodes compared to the strut-based lattices they were compared to.

Similar to quasi-static testing, it becomes apparent that the effect of topology has been thoroughly investigated for different strut-based and sheet-based lattices employing periodic unit cells. Still, no previous work has investigated how different topological alternations may affect the fatigue performance of stochastic lattice structures.

2.4. Additive manufacturing of lattice structures

2.4.1. Methods for lattice fabrication

Unlike traditional manufacturing methods that often involve subtracting material from a larger block, AM builds objects layer-by-layer, adding material only where needed. This approach allows for the creation of complex geometries that are often impossible to achieve with conventional methods, such as lattice structures.

The process begins with a digital 3D model, usually created using computer-aided design (CAD) software. This model is then sliced into thin horizontal layers using dedicated software. Conventionally, the axis along which the layer-by-layer building occurs is considered the Cartesian Z-axis. The slicing software further enables positioning a part in the X-Y plane, adding vertical elements to support any overhanging surfaces, and finally applying process parameters for manufacturing. After the printing process is completed, parts are removed from the AM machine and post-processed, which can include the removal of support material, cleaning, and surface finishing.

There are seven classifications of additive manufacturing methods, as defined by international standards (ISO 17296-2 and ISO/ASTM 52900 (**Figure 2.5**)). A brief description of each one is given below:

a) **Binder Jetting**

A liquid binding agent is selectively deposited onto a powder bed, binding the powder particles together to form solid layers. After each layer, a fresh layer of powder is spread, and the process repeats until the entire object is formed. The unbound powder serves as a support structure during printing and is later removed.

b) **Directed Energy Deposition**

The heat from an energy source (laser or electron beam) is directly applied onto a material feedstock (metal powder or wire) to melt it and deposit it onto a surface, layer by layer. This method is typically used to add material to existing parts (e.g. for repair) or create new parts from scratch.

c) **Material Extrusion**

It involves pushing thermoplastic filament through a heated nozzle, which melts the material. The printer head moves to deposit the material layer by layer, solidifying as it cools. Material extrusion is also referred to as Fused Deposition Modelling (FDM) or Fused filament fabrication (FFF).

d) **Material Jetting**

Droplets of material (usually a photopolymer) are selectively deposited onto a build platform and cured using UV light. Multiple print heads can be used to deposit different materials simultaneously, allowing for multi-material and full-colour printing.

e) **Powder Bed Fusion**

A high-powered energy source, such as a laser or electron beam, is used to selectively fuse powdered material spread on a plate/bed. The process is repeated layer by layer to build the object. Unfused powder acts as a support and is removed after the build is complete.

Depending on the material used, powder bed fusion (PBF) systems will fully melt the powder (metals) or partially melt it to result in sintering (polymers and ceramics). Due to this distinction, laser powder bed fusion systems are further subcategorised using commercially established names: Selective Laser Melting (SLM) and Selective Laser Sintering (SLS). In contrast, an electron beam is primarily used in metals; thus, the process is widely referred to as Electron Beam Melting (EBM).

f) **Sheet Lamination**

It involves bonding sheets of material together, layer by layer, using adhesives or ultrasonic welding. The sheets are precisely cut using a laser to shape, either before or after bonding, to form the final object.

g) **Vat Photopolymerization**

It involves curing a liquid photopolymer resin in a vat, layer by layer, using a light source. This can be a laser (Stereolithography) or a projector (Digital Light Processing). The resin solidifies where the light is focused, forming the object.

All the above have been previously used to fabricate complex or porous structures, however, powder bed fusion remains the most widely used in industrial applications owing to high precision (10s of μm) and versatile material selection – which are particularly relevant to orthopaedics (e.g., titanium, stainless steel, cobalt-chrome alloys). In terms of resolution, PBF outperforms Material Extrusion, Directed Energy Deposition, Sheet Lamination and Material Jetting which provide a resolution in the scale of 100s μm (178). Vat Photopolymerization, although can provide resolution as low as 0.1 μm using two-photon polymerisation (179,180), is typically limited to photopolymers that lack the mechanical strength required for load-bearing applications. Last, Binder Jetting, although capable of producing metal parts with high resolution (10s μm), requires post-processing steps like sintering or infiltration that can compromise the mechanical properties and precision of the final part due to dimensional changes.

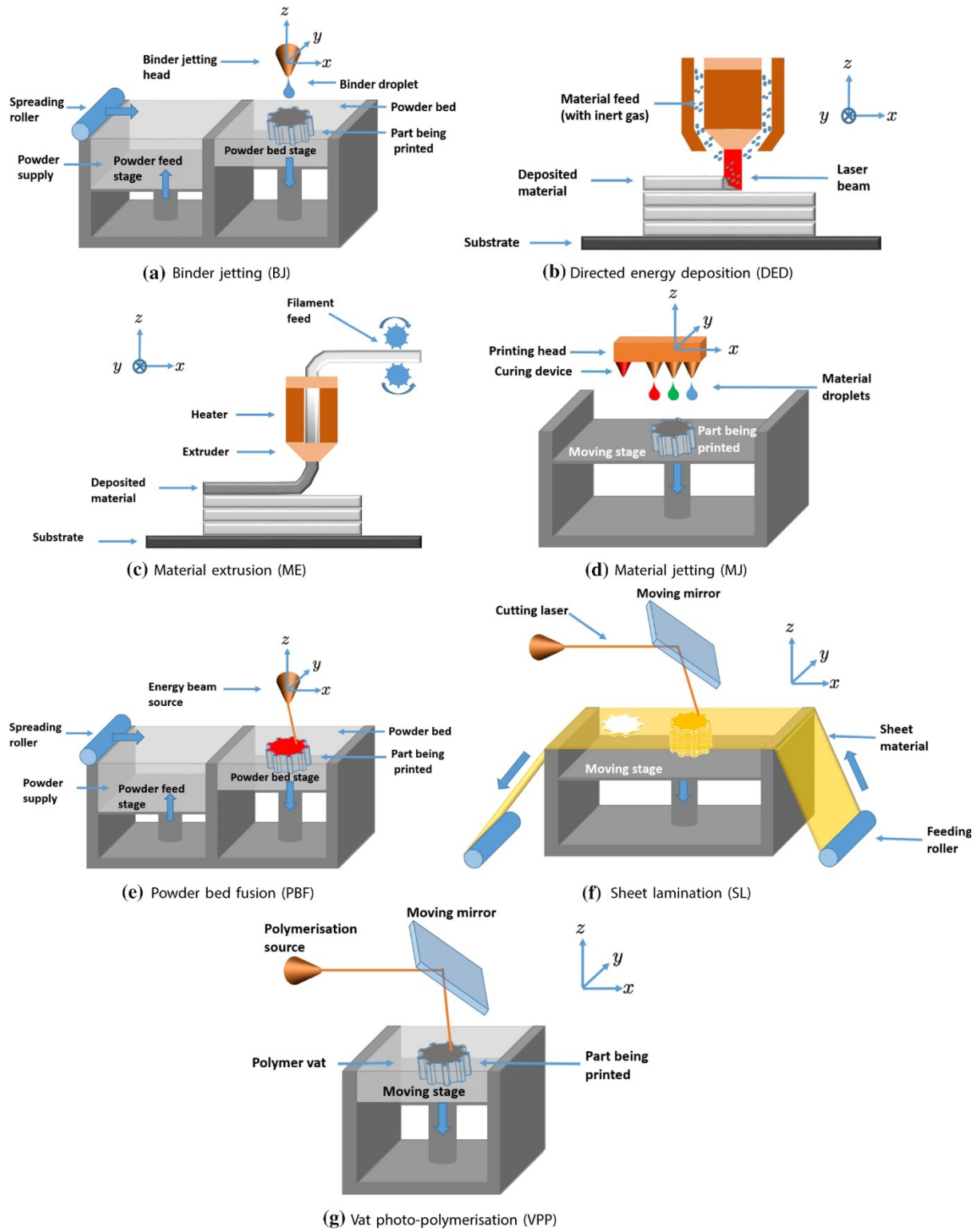


Figure 2.5. Illustration of the seven AM methods according to ISO/ASTM 52900. Figure reproduced from (181) with permission from Springer Nature (License No. 5975000936671).

2.4.2. Limitations in powder bed fusion

While highly accurate compared to other additive manufacturing methods, powder bed fusion is known to lead to defects affecting the quality and performance of the final products. Major defects include:

- Internal porosity, often caused by insufficient laser energy leading to incomplete melting of powder particles, or conversely, very high laser energy leading to splashing of the powder bed leading to entrapped gas or leaving voids. (182)
- Incomplete fusion, where an inadequate energy input or improper scanning strategy results in partially melted regions, compromising the mechanical integrity. (183)
- Surface roughness, manifested as semi-sintered particles (due to overheating), stair-stepping (i.e., visible ridges or step-like patterns formed on the edges of 3D objects due to the layer-by-layer printing process's inability to accurately represent sloped or curved geometries), waviness (due to overlapping melt-pools generated by the laser when binding the powder) (89,184)
- Residual stresses and warping, usually a product of thermal gradients and rapid solidification, where the differential thermal contraction induces tensile and compressive internal stresses that can cause cracks or part distortion. (185,186)

These defects arise from diverse factors like the energy density and melt-pool dynamics (i.e., size, shape, temperature gradient, mass flow and stability), the layer thickness, the adequacy of supports, powder particle size, and material properties. Among these, energy density and melt-pool dynamics are closely linked to laser parameters such as power, scanning speed, and firing duration. Extensive research on laser parameter optimisation has significantly reduced traditional defects like porosity and incomplete fusion in widely used materials such as Ti-6Al-4V and 316L stainless steel.

Current research efforts aim to extend these optimisation strategies to new materials, while also advancing multi-material fabrication capabilities (187,188). However, even well-established materials continue to exhibit challenges such as anisotropic mechanical properties due to directional solidification and residual stresses. To mitigate these limitations and enhance the efficiency of PBF systems, current research focuses on optimising scan strategies (e.g., hatching patterns), implementing real-time in-situ monitoring with artificial intelligence-driven adaptive control, coordinating multi-laser configurations for increased productivity, and developing novel post-processing treatments to improve part quality and mechanical performance (189–191).

Particularly in metals, the resulting microstructure and mechanical anisotropy remain persistent challenges during powder bed fusion. The rapid heating and cooling cycles (cooling rate $\approx 10^6$ K/s) lead to thermal gradients and non-equilibrium solidification (192). The resulting microstructure depends on the thermal gradient and solidification rate (**Figure 2.6**) and can be planar, cellular, and dendritic (193). In powder bed fusion, the thermal gradient is $\approx 10^7$ K/m and the solidification rate is ≈ 0.1 m/s which together with the high scanning speed form an elongated but shallow melt pool. Guided by the shape of the melt pool, the solidified microstructure displays a fine columnar (dendritic) grain structure that aligns with the build direction (Z-axis) (194). Eventually, this results in anisotropic properties, where the mechanical strength and ductility vary depending on the orientation relative to the build direction. Generally, horizontally orientated AM (solid) parts tend to have higher tensile/compressive strength under quasi-static and fatigue loading compared to vertically orientated parts (195).

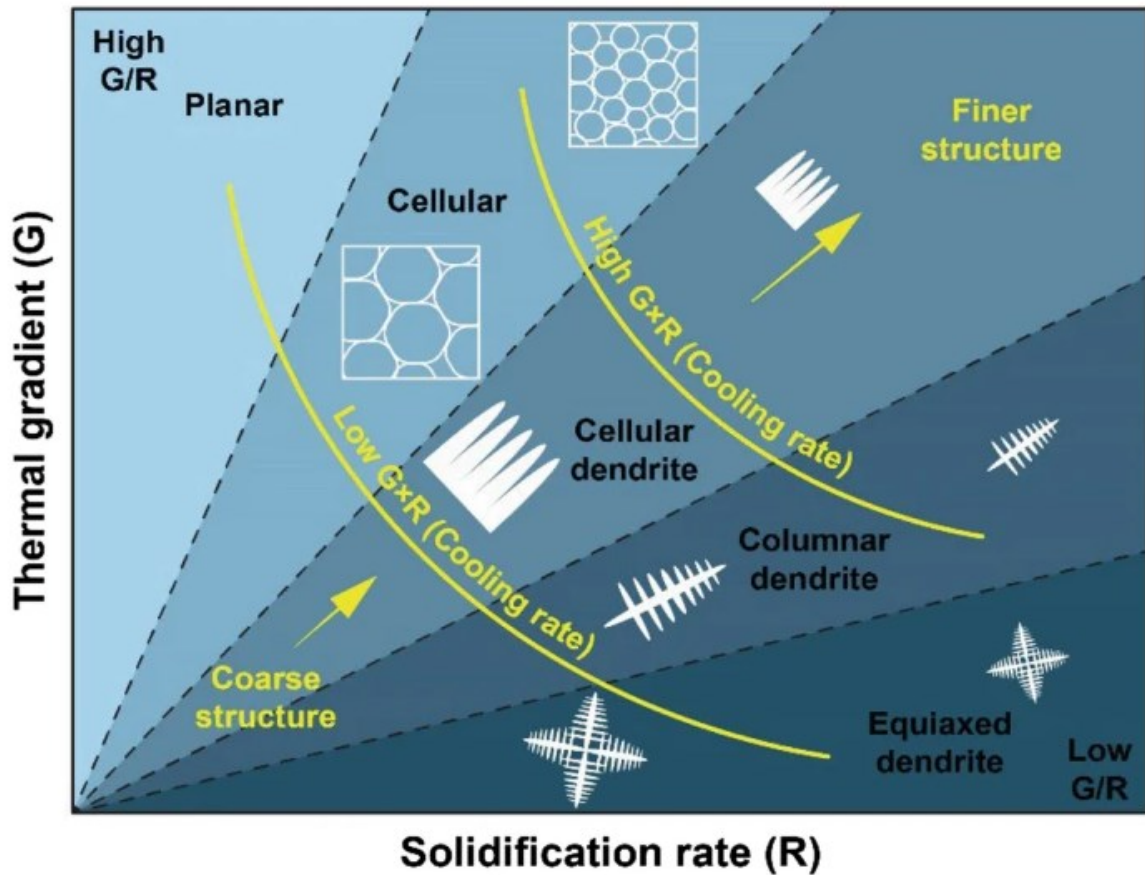


Figure 2.6. The effect of thermal gradient and solidification rate on material microstructure.
 Figures reproduced from (192) with permission from Springer Nature (License No. 5975001164421).

The coupled effect of structural defects and anisotropy are particularly pronounced in lattice structures. Interestingly, the mechanical properties of lattice structural elements (i.e., struts) often show trends that contradict those observed in bulk AM parts. Vertically built struts typically exhibit higher strength and elastic modulus, but lower ductility compared to horizontal struts for a variety of materials such as CP-Ti (196), Ti-6Al-4V (197,198), steel 316L (199) and Al-Si-10Mg (200). This contradiction between the behaviour of fine struts and bulk parts can be explained by the amplified influence of defects generated in low-angled struts.

Vertical struts are built on top of solidified areas from previous layers, which provide heat-conductive routes. On the contrary, low-angled struts are built partially on solid and powder regions, resulting in slower heat dissipation (89). This slower cooling leads to overheating in the vicinity of low-angled struts, causing high surface roughness in the form of semi-sintered particles and dross at downward-facing surfaces of overhanging struts (201,202). Additionally, low-angled struts are more prone to the stair-stepping effect which can result in poor overlap between consecutive melt-pools and imperfect layer bonding, compromising mechanical strength. Last, vertical struts, being directly aligned with the build direction, may experience more uniform cooling, leading to finer microstructures (203). Finer grains or more uniform microstructures can increase strength but reduce ductility, making the material more brittle.

Overall, the sensitivity of AM struts to defects is also associated with their inferior strength and elastic modulus compared to their respective base (wrought) material, as seen in cases of Ti-6Al-4V and 316L stainless steel (199,204).

Last, a major drawback in reproducibility in powder bed fusion systems concerns powder feedstock. Reusing powder for new builds is a common practice. However, it can greatly comprise the dimensional accuracy and the mechanical performance of AM parts. Reused powder exhibits roughened particle surface, aggregations where small (satellite) particles bind to larger ones, and often chemical contamination (impurities and oxidation) (205). These issues can lower powder flowability and composition, ultimately leading to higher surface roughness and inferior mechanical properties when AM parts are made using reused powder compared to virgin powder. The effect of powder reuse has also been seen in lattice structures, where lower static/fatigue strength and elastic modulus were recorded for increasing powder reusing cycles, in cases of tantalum (206) and CP-Ti (157).

2.4.3. Distinguish between powder bed fusion systems

Key differences are found between the two types of metal powder bed fusion systems: SLM and EBM. SLM generally offers higher resolution and finer detail due to the laser's smaller spot size (10s versus 100s μm) (207). However, the high energy density of the laser greatly increases the surface roughness of SLM parts compared to EBM parts. An interesting example comes from the implant industry where acetabular cups exhibited ≈ 8 times more semi-sintered particles/ mm^2 when built using SLM compared to EBM, however, the exact combination of process parameters used was not provided in the study (208). Another major challenge in powder bed fusion is the need for a controlled environment that will minimise the introduction of impurities in the fabricated parts. During SLM, the building chamber is filled with an inert gas, such as argon or nitrogen, which prevents the powder bed from chemically reacting (e.g., oxidation) with the environment. On the contrary, EBM operates in a vacuum and involves a preheating step that reduces thermal gradients, and the material solidification rate compared to SLM. This generally results in lower residual stresses and fewer defects like warping.

The high differences in cooling rates (typically 10^4 - 10^6 K/s in SLM and 10^3 - 10^5 K/s in EBM) also result in SLM parts exhibiting finer microstructures which can have contradictory performance under quasi-static or cyclic loading (209). In (210) solid Ti-6Al-4V specimens were fabricated with SLM and EBM, the SLM parts exhibited a martensitic microstructure resulting in 30% higher tensile strength but 50% less ductility than the EBM parts with lamellar grain morphology. In (211), the same lattice structure was found to have $\times 10$ times more entrapped pores when fabricated using SLM than using EBM. This had a limited effect on quasi-static and high-cycle fatigue strength (low-stress levels), but greatly reduced low-cycle fatigue life (high-stress levels).

Overall, each method exhibits advantages and disadvantages and choosing between them is primarily a matter of the material being used and design requirements. For example, EBM allows the process of very brittle metals that would be prone to cracks due to the fast solidification of SLM. At the same time, it poses a limitation on material selection, as not all metals can withstand the vacuum due to sublimation and degradation, especially when containing constituents such as Zn, Mg, and Pb (209).

2.4.4. Process control in laser powder bed fusion

The slicing results in 2D imprints (i.e., the contour) of a 3D part at different layers, and during the powder bed fusion, a firing laser typically hatches the contour of each layer to produce the final 3D part. The major parameters employed during laser powder bed fusion are shown in **Figure 2.7** and constitute the following:

- Laser power (10s to 100s W),
- Layer thickness (10s of μm),
- Hatching spacing (10s of μm),
- Distance between consecutive firing points (10s of μm),
- Exposure time of laser (10s to 100s of μs).

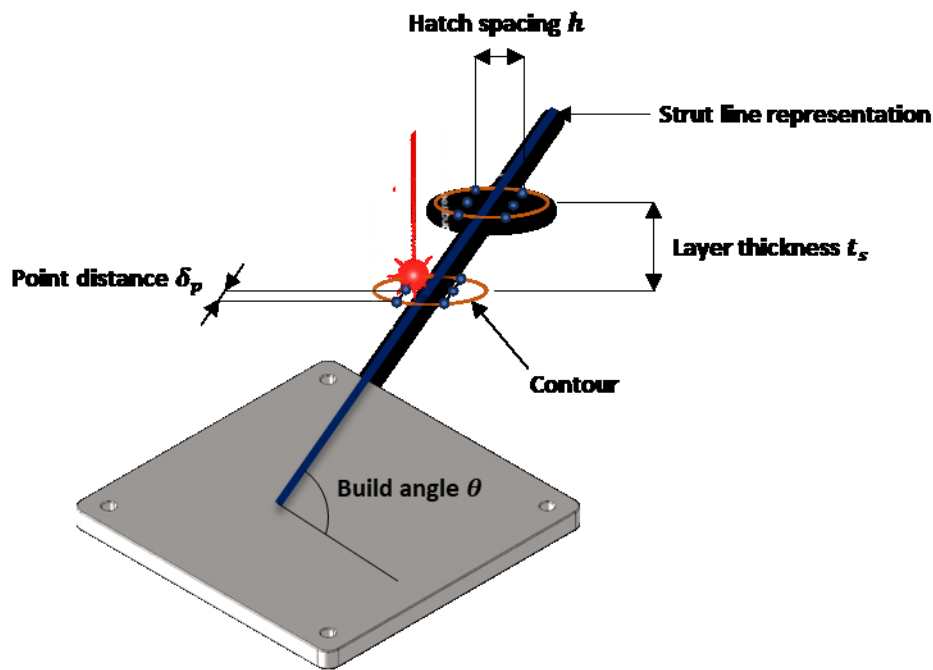


Figure 2.7. The major laser parameters used in powder bed fusion. *The illustration presents the fabrication of struts as done in the current work by representing them as lines.*

Analytical expressions for the energy transmitted to the powder bed are typically used to select process parameters for balancing printing outcome and productivity. The volumetric energy density [J/mm^3] is the most widespread physical entity used in powder bed fusion:

$$ED = \frac{P}{v \cdot h \cdot t_s} \quad \text{Equation 2.9}$$

The expression combines laser power P , scan speed v (1s to 1000s of mm/s), hatch spacing h and layer thickness t_s . While scan speed is assumed equal to:

$$v = \delta_p / \delta_t \quad \text{Equation 2.10}$$

Where, δ_p being firing point distance and δ_t being the laser exposure time.

A limitation of this expression is that it provides a magnitude of energy used without incorporating material-specific information. Still, as with any manufacturing process, the powder material is detrimental to the choice of process parameters in powder bed fusion. Properties like thermal conductivity and coefficient of thermal expansion affect the precision, surface finish, and potential for defects like warping. Thus, a more accurate physical model should correlate process parameters and material properties to provide insights into the printing process.

The specific enthalpy ΔH [J/kg] (89,212) has been proposed as a model to estimate the size of the melt pool as a function of laser power P , laser spot diameter Φ (10s of μm), scan speed v , as well as powder material properties (density ρ , absorptivity A , thermal diffusivity D):

$$\Delta H = \frac{A \cdot P}{\rho \sqrt{\pi D v \Phi^3}} \quad \text{Equation 2.11}$$

This expression on the other hand does not include the layer thickness and hatch spacing, meaning that individual models should be used for different values of the above parameters. Still, it has been shown that melt pool size – which depends on material, power and scanning speed, layer thickness and hatch space collectively affects the internal porosity due to lack of fusion (213,214). In this regard, optimising the powder bed fusion process while keeping layer thickness and hatch spacing fixed appears the most efficient path. Making the selection of an appropriate combination of power and scanning speed central aim for successful printing with low roughness and entrapped porosity. Although the exact values of these parameters may vary depending on the specific experimental setup, powder material and parameters used (89,212,215–217), a generic classification can be done as follows:

- High power and low velocity, resulting in high enthalpy, will lead to an enlarged melt pool. Although this can fortunately increase the overlap of melt pools when hatching each layer or the overlap between successive layers, it will also increase the energy density. High energy densities may cause evaporation or ablation of powder and a turbulent melt pool due to excessive heat. These can eventually lead to denudation zones, keyhole porosity, as well as open porosity and roughness on the top surface due to splashing or “balling” of molten powder.
- Low power and high velocity, resulting in low enthalpy, will generate insufficient heat for the complete melting of powder and will lead to reduced melt pool size with decreased overlap. As a result, pores and semi-sintered powder particles can be left on the top printed surface.

These observations were first recorded for bulk AM parts, but highly correlated with observations in the case of lattice structures. High laser power allows the fabrication of large strut diameters; however, it typically introduces a higher percentage of entrapped pores (89,218). Increasing laser scanning speed will lead to smaller strut diameters, interestingly intermediate speeds (4000 mm/s) were found to exhibit higher internal porosity compared to low (1000 mm/s) or high (7000 mm/s) speeds (218).

All the above suggests that a conscious combination of low power and low speed following the selected layer thickness and hatching spacing should result in fine struts with low entrapped porosity and sufficient melt-pool overlap for structural integrity.

2.4.5. Process parameters and fatigue performance

Choosing appropriate printing parameters to achieve “optimum” printing outcomes without over-melting or under-melting has an apparent influence on surface roughness and entrapped porosity. These two defects are known to critically affect the mechanical performance of an AM part, particularly the high-cycle fatigue strength (219). Apart from laser power and scanning speed, there are additional parameters specific to laser powder bed fusion that appear to have different influences on the fatigue performance of lattice structures.

Increasing layer thickness leads to a high production rate with a compromise of dimensional accuracy due to higher surface roughness. Still, fatigue strength was found to be independent of the layer thickness for CoCr lattice structures built with a layer thickness of 30 or 60 μm (159).

Scanning strategy on the other hand appeared to influence fatigue performance in lattice structures made of CP-Ti, Ti-6Al-4V, Ta, and Ti-Ta (114). Generating lattices by hatching the contour of their struts led to 8% higher fatigue strength at 10^6 cycles compared to structures whose struts were generated by firing a single point with a continuous or pulsed laser exposure. Further, Karami et al (161) compared the use of a continuous and pulsed laser for the fabrication of Ti-6Al-4V lattices. They reported that the continuous laser produced structures of coarse microstructure and higher fatigue strength due to decreased internal porosity and semi-melted particles, while pulsed laser provided better quasi-static properties due to a more homogenous and finer microstructure.

2.4.6. Post-processing for mechanical performance

Defects such as semi-sintered particles, residual stresses, internal porosity, and microstructural heterogeneity are particularly harmful to fatigue strength as they can lead to stress concentrations and become crack initiation sites during cyclic loading (158,220). Post-processing treatments are utilised to rectify such defects and ameliorate the mechanical performance of additively manufactured parts.

The most widely used post-processing treatments for powder bed fusion parts concern surface and heat treatments. Surface treatments aim at enhancing the surface finish with or without material removal, and they comprise mechanical methods (e.g., machining, media blasting, vibratory finishing), chemical methods (e.g., chemical etching, electropolishing) and laser-based methods (e.g., laser micro-machining, laser shock peening) (221). In contrast, thermal treatments are applied to release any residual stresses or to induce microstructure changes within a part.

Surface treatments

Media blasting (e.g., sandblasting) and vibratory finishing are the most common mechanical methods to smoothen the surface of additively manufactured specimens. However, in the case of lattice structures, the abrasive media used in such methods cannot typically reach the core of the specimens owing to the complex porous architecture. The same limitation occurs also in the case of laser-based methods where the laser cannot reach regions in the core of the part. Hence, chemical-based methods are more suitable for surface finishing of AM lattice structures.

Chemical etching is the most widely used surface treatment applied in lattice structures. A chemical reagent reacts with the AM substrate leading to material removal through controlled corrosion. Typical reagents constitute strong and highly corrosive acids like nitric acid, sulfuric acid, and hydrochloric acid. Since relying on a fluid media, chemical etching has been successfully used to remove semi-sintered particles from lattice structures. This process reduces the surface irregularities and stress concentration sites, albeit it often introduces pits on the surface due to the removal of the semi-sintered particles (159). More importantly, chemical etching is inevitably accompanied by a reduction in strut or wall thickness and eventually in relative density, which becomes more apparent for prolonged times of etching (222,223). Lower relative density results in drastically reduced static and fatigue strength. Thus, the absolute S-N curves in fatigue testing of the initial (as-built) specimens appear higher than the chemically etched specimens. If normalised stress values are used, the chemically etched specimens appear higher. This has been previously shown in lattices made with Ti-6Al-4V (224) and CoCr (159,225). This is attributed to the significantly higher tensile strength of etched struts compared to as-built struts. For the same apparent strut diameter, a larger portion of the strut can effectively carry a load in etched

than in as-built struts, owing to lower waviness and semi-sintered particles that do not contribute to the strength (226).

Although a well-established surface treatment, conventional chemical etching relies on immersing the parts on the acid solution. This introduces limitations when using large-sized lattice structures, as the acid cannot penetrate deeply or evenly inside the lattice. This leads to uneven etching, with the material removal being more aggressive at the outer regions and less effective near the core of the lattice. Applying a fluid flow in the etchant is also avoided as it can induce even higher heterogeneities in the material removal.

To address these challenges, hybrid chemical–electrochemical surface treatments have been developed to combine hydrodynamic flow and electrolytes capable to remove large-scale roughness (10s-100s of μm) that typically cannot be removed with conventional electropolishing (221). Such methods have successfully applied to Ti-Al-4V lattice structures, resulting in 80% increase of normalised fatigue strength compared to untreated structures (227).

Thermal treatments

Heating for stress relief in the case of lattice structures is more relevant when they are combined with solid sections within the same component. Solid parts are more prone to warping or cracking owing to residual stresses. In contrast, lattice structures do not experience residual stresses since struts are unconfined and free to deform during the manufacturing process (228).

High-temperature annealing is traditionally employed for alloyed metals to induce a more ductile behaviour. Some representative examples are found in the case of Ti-6Al-4V. In (229), meshes were annealed at sub-transus temperatures: 750, 850 and 950°C to transform the as-built martensite α -phase to coarse lamellae which improved structures' ductility, and eventually fatigue life. While in (230), stochastic lattices were annealed at 920°C and super-transus temperatures of 1050 and 1200°C. Super-transus temperatures were found to be more beneficial, leading to a 15% increase in the elastic modulus and a 75% increase in the fatigue strength of the heat-treated structures compared to the as-built condition.

Hot-isostatic pressing (HIP) provides a combination of microstructure alternation to more favourable coarser grains and an increase in specimen density due to pores closure (228). Many published works have shown that using HIP, especially combined with chemical etching, can lead to a significant increase in the fatigue strength of lattices (159,161,224).

2.5. Lattice structures as scaffolds for bone regeneration

Until now, this review has highlighted how the base material, relative density (or porosity), and topology dictate the mechanical behaviour of a lattice structure. However, when employed in orthopaedic implants, lattice structures should combine material properties and topological characteristics that not only provide beneficial mechanical properties but also induce favourable biological function on the tissue and cells that host the implant.

2.5.1. Preclinical models for biological evaluation

Employing synthetic constructs with mechanical properties that mimic the ones of natural bone has been shown to induce higher bone in-growth and enhance bone mineralisation compared to stiffer alternatives that “shield” bone loading. This finding has been well-supported by scientific evidence using

preclinical models such as *in vivo* studies in animals (39,231,232) or *ex vivo* studies where a bioreactor setup allows the preservation of isolated animal or human tissue by supplying nutrients using circulating flow, often in combination with applied loading to stimulate tissue remodelling (233–235).

Before progressing to these large models, seeding an implant substrate with tissue-specific cells and culturing them *in vitro* serves as a key preliminary model for initial biological evaluation. This relatively simple approach allows the assessment of the interaction between the implant and cells, providing valuable information on major biological processes such as cell adhesion, cytotoxicity, cell proliferation, differentiation, and, ultimately, tissue formation. This occurs in a controlled environment, free from the interference of multiple external factors that are present *in vivo*.

In the context of bone regeneration, typical cell models include cell lines like the MC3T3-E1 (derived from mouse calvaria) and MG-63 (derived from human osteosarcoma patients) as well as primary cells like mesenchymal stromal cells which are isolated from human or animal donors². *In vitro* experiments are usually performed using a single cell type (i.e., monoculture) under static conditions. However, more advanced models can also arise by co-culturing different cell types, such as osteoblasts with osteoclasts (236,237) or endothelial cells (238,239), or utilising bioreactor setups. These approaches more closely mimic the multicellular and multi-stimulus *in vivo* environment, while also enhancing cellular activities such as differentiation and mineralisation (240).

2.5.2. Material selection

The material that an implant is made from determines its mechanical properties and biocompatibility. Properties, such as elastic modulus, yield strength, fatigue strength, corrosion and wear resistance not only define the implant's mechanical performance but also its compliance with the environment where the implant is placed. From a biological perspective, the base material must be biocompatible for the organism, meaning it can stimulate osseointegration without, however, being harmful to the body which would activate immune response mechanisms (19).

Metals are the most widely used materials for orthopaedic implants and devices owing to their excellent manufacturability and favourable mechanical properties.

Titanium and its alloys are historically the most commonly used materials for AM porous structures due to their biocompatibility, high specific strength, low elastic modulus and good corrosion resistance. Titanium-based implants demonstrate sufficient cell adhesion, cell proliferation and bone in-growth (39,241). CP-Ti is advantageous due to its ductile nature (for press-fit applications), absence of toxic alloying elements (242,243), while as a single-phase metal, CP-Ti demonstrates higher electrochemical corrosion resistance compared to alloys (244). However, Ti alloys – with Ti-6Al-4V being the most prominent – remain the golden standard in the orthopaedic industry due to their well-established manufacturability (e.g., extended use in welding), ample supply of AM powder, as well as the heat treatment compatibility due to the additional β -phase. Proper control of the alloy microstructure and heat treatment can lower their stiffness and improve their ductility while maintaining high strength (230,245). CP-Ti and Ti-6Al-4V show similar osseointegration potential, thus CP-Ti is typically preferred

² Primary cells are isolated directly from tissues and, while they exhibit a relatively low lifespan (they can only divide a certain number of times), they better mimic the *in vivo* environment, making them biologically more relevant. In contrast, cell lines originate from primary cells but are modified to divide (i.e., proliferate) almost indefinitely. This is often achieved via gene mutations, the introduction of oncogenes, or because they derive from a tumorigenic source. In biological experiments, they offer greater consistency, reproducibility, and ease of handling, but they may diverge from their original *in vivo* characteristics over time due to genetic changes.

for dental implants due to its superior corrosion resistance, while Ti-6Al-4V is chosen for orthopaedic implants for its higher load-bearing capacity (246).

Besides titanium and its alloys, cobalt–chromium–molybdenum (CoCrMo) alloys, stainless steel, and tantalum are widely used in orthopaedics due to their distinct mechanical and biological properties. Cobalt-chromium alloys are commonly utilised in joint replacements owing to their excellent wear resistance and strength. Stainless steel (particularly 316L) is often employed in temporary implants like fracture fixation devices because it offers good corrosion resistance and low cost. However, its use in joint arthroplasty has diminished over time because it is less biocompatible and corrosion-resistant compared to modern alternatives (247). Tantalum is known for being highly biocompatible, ductile, and corrosion resistant owing to its ability to form a dense oxide layer, making it a contemporary choice in porous implants for bone in-growth, particularly in spinal fusion and acetabular cups (248).

These metallic materials undergo negligible corrosion and hence will remain permanently installed in the body unless removed due to infection or implant failure. In this regard, magnesium and zinc alloys have recently attracted significant scientific interest as they are biodegradable and can gradually degrade inside the body, eliminating the need for secondary removal surgeries. Both materials offer excellent biocompatibility and low stiffness, promoting bone regeneration during degradation. Moreover, recent advancements in additive manufacturing have enabled the development of zinc/magnesium-based lattice structures, making them promising alternatives for orthopaedic applications (249–251).

Nevertheless, even the most well-established metals in orthopaedics face great drawbacks. Released metal ions or particles due to corrosion or wear of alloyed materials – mainly cobalt from CoCr alloys, nickel from stainless steels, and vanadium from Ti alloys (252) – can lead to perilous conditions such as allergic or inflammatory reactions, blood poisoning (metallosis), cytotoxicity, carcinogenesis, and genotoxicity (241,253). Even pure metals, such as CP-Ti and Ta, can in certain cases be toxic (242). However, these materials typically form dense oxide films in contact with air, which are bioinert and in most applications protect the implants from corrosion (19).

In the case of patients with metal sensitivity or severe allergic reactions due to metallic debris release (owing to metal-on-metal contact), revision surgery is performed to replace the metallic components with ceramic-based implants (254,255). Although these materials are highly biocompatible, “hypoallergenic” and offer load-bearing capacity, their brittle nature still hinders their extended use in joint arthroplasty due to sensitivity to multi-axial cyclic loading.

2.5.3. Scaffold architecture

From a biological perspective, scaffolds should have interconnected open pores with size and shape that favour bone tissue formation and maturation for superior implant-to-bone fixation (256). Small pores increase the scaffold’s surface area-to-volume benefit initially cellular adhesion and later cell communication towards rapid differentiation, tissue formation and mineralisation (257,258). Large pores, on the other hand, result in high fluid permeability allowing deep cell migration, oxygen and nutrient transportation, aiding tissue in-growth and vascularisation in large-sized implants (257,259,260). Pore sizes between 300 μm and 1200 μm satisfy these criteria while avoiding phenomena such as precocious pore occlusion due to very small pores (257) or poor tissue formation and mineralisation due to very large pores (261). A pore size should not be smaller than 100 μm , otherwise, it cannot allow the penetration of thick mineralised tissue other than thin fibrous tissue, while in extreme conditions it can be comparable to the size of cells (10s μm) and hinder cell migration (262,263), while many studies conclude that pore sizes of approximately 600 μm optimise scaffold rheological and

biological properties (261,264). Still, a holistic review of the literature suggests that an optimal pore size highly depends on pore geometry and topology (19,263,265).

Lattice topologies where struts form acute angles (i.e., triangulated pores) have been found to accelerate cell differentiation compared to topologies with obtuse-angled or circular-shaped pores (257,266,267). While studies in channels of different shapes report that small radius of curvature and the presence of concavities greatly accelerate tissue growth (268,269). This is typically attributed to the curvature-driven tissue formation, where tissue preferably grows in concave regions of 3D environments like pore corners (268). The corners, where struts or walls meet, are areas of maximum curvature where stresses concentrate. Cells and tissue in these regions experience increased surface tension due to the high curvature, new ECM is formed to bridge the corners and increase the radius of curvature (270). Cells lying along the strut length where the curvature is zero, are stimulated when their neighbourhood becomes curved due to tissue growing outward from the corners, as more cells sense and bind to the ECM the pores are progressively occluded (271). Cell proliferation and differentiation are driven by local forces that cells sense from substrate geometry, while smaller pores typically accelerate the rate of tissue formation (269,272,273). Thus, scaffold design has always focused on tuning pore size and geometry to provide physical cues for beneficial cell motility and communication (274,275).

AM implants that are commercially available and currently used in clinical practice employ thin layers (1-2 mm) of lattice structures at the implant-bone interface to induce osseointegration. Lattices are primarily used in artificial joints for the hip (acetabular cups and femoral stems), the knee (femoral, tibial, and patella components) and the shoulder (glenoid component and humeral stem). The lattice types used are predominantly stochastic structures, with periodic structures being less common. The average connectivity is typically close to 4, aligning with that of natural trabecular bone (3 to 5 trabeculae per node) (276). The mean pore size of these structures typically ranges between 300 to 650 μm , strut thicknesses range from 230 to 400 μm , and the resulting porosity ranges from 60% to 75% (277–279).

Overall, stochastic structures better mimic the random micro-architecture of trabecular bone and have been shown to favour cellular behaviour over periodic lattices of similar pore size or surface area (26,258,280). Limited data exist regarding how stochastic lattices affect osseointegration, whereas existing studies have focused solely on the influence of pore size (281,282). Yet, pore size typically varies inside such heterogeneous structures compared to periodic lattices that demonstrate a more confined distribution (90). Thus, pore size might not be adequate for controlling cellular behaviour in stochastic designs.

2.5.4. Post-processing for osteoconductivity

Grafts for bone regeneration should combine two major biological characteristics: osteoconduction and osteoinduction (16). Osteoconduction refers to the ability of the material to support cell adhesion, proliferation, matrix formation, and ultimately, mature bone formation. Osteoinduction refers to the ability of a material to induce bone formation when implanted even at sites where bone is not normally found (heterotopic sites) (283). Yet, of these two, osteoconduction is more relevant in the context of cementless implants for joint replacement.

Post-processing techniques can be applied to introduce bioactivity in implants, and they refer to physical methods, chemical methods, and biomimetic coatings (19). In most cases, these methods aim at activating the implant surface to exhibit chemical reactivity or physical characteristics that benefit cell function.

Physical methods

Key examples of physical post-processing methods are media blasting, polishing, laser engraving, plasma treatment and heat treatment.

The first two mechanical treatments aim to introduce micro-scale roughness that benefits osteoblast adhesion, proliferation, and differentiation. This is investigated using the surface roughness parameters Ra (average roughness) and Rz (average maximum height of the profile). Ra values around 1-2 μm (284–287) and Rz values between 1-10 μm (288) have been shown to favour cell adhesion and protein adsorption due to the high surface area and wettability and stimulate osteoblastic differentiation due to the introduction of surface discontinuities comparable to cell size ($\sim 10 \mu\text{m}$).

Nanotexturing achieved through laser engraving is another method to enhance cell adhesion by increasing surface wettability, while also introducing nano-patterns that cells can sense, leading to adjustments in their morphology and orientation towards an elongated shape, which favours differentiation (289,290).

However, mechanical treatments have two major limitations. First, they can introduce contaminants by leaving residual particles on the implant surface (291). This issue is particularly associated with sandblasting, leading to its replacement by cleaner alternatives such as dry ice blasting (i.e., carbon dioxide), where no harmful chemicals are used (292). Second, regardless of the blasting medium, these methods are primarily applied to solid implants, as the complex architecture of 3D lattices does not allow homogeneous treatment throughout the structure.

Plasma treatment and heat treatment are more suitable for lattices, while they have also been extensively used in solid implants in clinical practice (293,294). These treatments are typically used in metals like titanium to enhance their bioactivity by creating a thick oxide layer (e.g., titanium dioxide, TiO_2). This oxidation layer demonstrates a nanostructure topography that increases the surface hydrophilicity and thus enhances protein adsorption and cell adhesion (295). More importantly, it gives the metal corrosive resistance and chemical reactivity (296). The latter is crucial for the osseointegration of metallic implants as it allows the formation of bone-like apatite (e.g., hydroxyapatite) on the surface of the implant due to the reaction of the oxidised layer with the Ca^{2+} and PO_4^{3-} ions found in body fluids (297).

Chemical methods

The application of anodisation and etching with acids or alkaline solutions are also used to enhance the hydrophilicity and bioactivity of metals by creating a nanostructured oxidised layer (298). Acids and alkaline solutions can improve the chemical reactivity of the surface by removing contaminants and impurities, while in some cases also introducing micro-roughness due to etching (299). Typical etchants constitute strong acids (e.g., HCl) and strong alkaline solutions (e.g., NaOH), while the combination of chemical and heat treatment has been shown to further enhance implant bioactivity. As a comparison to physical methods, the combination of HCl and heat treatment on titanium implants has been shown to enhance early *in vivo* bone formation compared to sandblasted or untreated samples (300). In addition, NaOH-heat-treated implants have been shown to induce stronger bone-implant bonding compared to untreated implants made of pure and alloyed titanium (301). This is again attributed to the oxidised titanium layer that promotes mineralised bone tissue formation, without the intervention of fibrous tissue typically seen in untreated implants. However, a further improvement in bioactivity has been noted when NaOH-heat-treated implants are further treated with HCl to dissolve the released sodium ions introduced due to the oxidation (302).

As previously discussed, chemical etching with highly corrosive acids (such as hydrofluoric and sulfuric acid) is often used in additively manufactured lattice structures to smoothen structure surfaces for better fatigue performance. In addition, though, chemical etching has been shown to increase the wettability of individual struts which is key for cell adhesion (303). While *in vitro* cell cultures have shown that chemical etching of Ti-6Al-4V scaffolds was able to promote osteogenic differentiation of mesenchymal stromal cells compared to untreated scaffolds (223).

Biomimetic coatings

A last method to increase bioactivity in metallic implants is applying surface coatings that mimic the biological environment.

Hydroxyapatite [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] and β -tricalcium phosphate [$\text{Ca}_3(\text{PO}_4)_2$] have been widely used as porous grafts or as a coating in clinical practise for the past decades to enhance the osseointegration on metallic implants. They can be used separately or combined, and they are typically applied via plasma spraying, electrodeposition, or sol-gel methods (304–306). They are typically used in solid press-fit implants that rely on on-growth, and they have been proven to be reliable for promoting mature bone tissue formation compared to untreated implants that are prone to fibrous tissue formation (305). While *in vivo* data suggest that these two coatings perform equally (305,307), synthetic hydroxyapatite can take years to biodegrade within the body, while β -tricalcium phosphate is generally resorbed within a few weeks after implantation (308). Still, an ongoing limitation of both ceramic coatings is their low bonding strength with metals which typically leads to cracking and delamination while implanted, leading to inflammation reactions due to the wear particles (309).

Alternative forms of coatings are the immobilisation of ECM products on metal surfaces. First, collagen type-1, which is the primary component of bone matrix, can be applied through various methods such as physical adsorption, dip-coating, and covalent bonding and serve as a biomimetic matrix that supports cell adhesion and encourages the formation of new bone tissue around the implant (310,311). Glycoproteins such as fibronectin and vitronectin can be applied in similar ways aiming to enhance osteoblast attachment (312). Last, peptides that mimic specific cell-binding motifs can be chemically grafted or immobilised onto the implant promoting targeted interactions with cell surface receptors. A typical example is the Arg-Gly-Asp (RGD) sequence which binds to integrin receptors of cells and aids cell adhesion (313,314). The advantage of peptides is that they can be designed to target specific cell surface receptors and promote tailored cell functions. In this context, previous works have shown how different peptides can be grafted on the same implant surface to induce cell adhesion, antimicrobial activity, osteogenesis and angiogenesis (315–317).

3. Aims and objectives

Additive manufacturing has enabled the fabrication of lattice structures with controllable micro-architectures and mechanical properties. Modern implants can employ lattice structures for stronger fixation through osseointegration, with a high potential for improving patients' quality of life. An ideal implant should combine (i) bone-matching mechanical properties to allow positive bone remodelling after surgery, (ii) an osteoconductive surface to benefit cell adhesion and tissue formation, and (iii) a micro-architecture to drive mature bone tissue formation from the early weeks after implantation.

Laser powder bed fusion is the current state-of-the-art technique for fabricating complex lattice structures with high resolution and a wide range of compatible base materials. Metal alloys like Ti-6Al-4V are considered the gold standard in the orthopaedic industry due to their high load-bearing capacity and favourable mechanical properties after heat treatment. However, single-phase metals such as CP-Ti offer important advantages, including natural flexibility essential for press-fit applications (without the necessity for heat treatment), high corrosion resistance due to a thick protective oxide layer, and lower cytotoxicity risks, as they lack harmful alloy elements like vanadium. These benefits have motivated this thesis to focus on lattice structures made exclusively of CP-Ti, which has received comparatively less attention than alloyed alternatives. Considering the significant role of lattice topology in mechanical behaviour, novel lattice designs could enable the development of load-bearing structures made from such flexible metals.

This work aimed to address the abovementioned challenges and suggest new approaches for the development of lattice-based orthopaedic implants that fulfil key biomechanical requirements.

First, the broad range of mechanical properties required for this application is a challenge. Previous research has primarily focused on periodic lattice structures. However, using periodic structures requires examining a range of unit cell topologies, each of which requires separate characterisation for different unit cell sizes and strut thicknesses. An alternative approach is to use a stochastic lattice structure, where a single relationship between the lattice design parameters and resulting mechanical properties should be possible. Three design parameters were used to define a stochastic structure: nodal connectivity, strut density and strut thickness. The first part of this work (**Chapter 5**) aimed to test two hypotheses:

- a) In analogy to periodic lattices, connectivity should be a key parameter to tune the topological characteristics of stochastic lattices. Hence by increasing connectivity, the stochastic lattice should transition from a compliant bend-dominated state to a rigid stretch-dominated state.
- b) This transition should also be reflected in fatigue strength. High connectivity should lead to stochastic structures which combine high porosity, bone-matching properties and better load-bearing capacity which are detrimental for bone replacement.

Second, integrating lattices in bone replacement implants requires accurate characterisation and prediction of lattice mechanical behaviour. Mechanical characterisation is done using specimens with standardised dimensions. The size of the specimen is known to affect the measured properties, but less is known about the role of aspect ratio (height-to-width ratio). A problem arises because (a) we know that the measured mechanical properties of a lattice depend on the aspect ratio of the specimen being tested and (b) the aspect ratio and size of implants can be wholly different to the standardised aspect ratio of test specimens used to characterise the lattice properties. A further challenge arises due to the

paucity of data on how topology interacts with aspect ratio to affect the lattice's mechanical properties. The second part of this work (**Chapter 6**) aimed to bring a better understanding of how aspect ratio in junction with size and topology affects the properties of a lattice, which is critical for integrating lattice structures in mechanical components of different scales.

Third, while it is recognised that stochastic structures bring together traits that favour their use in orthopaedic implants (i.e., biomimicry, isotropy, and shape conformity), no previous research has disclosed malleable ways to tailor their micro-architecture for better biological performance. While the vast majority of implants currently used in clinical practice employ stochastic lattices, existing scientific research primarily focuses on controlling the mechanical and biological performance of periodic lattices by adjusting pore size and shape. Still, less is known about how we can control the performance of stochastic lattices through their design parameters: nodal connectivity, strut density and strut thickness. The third part of this work (**Chapter 7**) aimed to answer this question through two *in vitro* experiments looking at the effect of design parameters on the activity of bone cells.

- The first experiment focused on connectivity and strut density which are detrimental for the micro-architecture. Pore size and spatial concavities stand out as key factors that benefit bone tissue formation. Consequently, the central hypothesis was that high connectivity lattices could combine biomimetic stiffness and high mechanical performance with suitable pore sizes and multiple corners to help mature bone regeneration inside porous implants.
- The second experiment focused on strut thickness, a topological feature which greatly influences porosity and strength. Preference towards thin or thick struts should be instructive when choosing the other two design parameters for balanced mechanical properties and bone ingrowth capacity.

A final concern is the poor surface finish of lattice structures fabricated using laser powder bed fusion, which is known to compromise cyclic endurance. Various surface treatments have been applied on Ti-6Al-4V and Co-Cr lattice structures and has been shown to improve fatigue strength and wettability which is crucial for osseointegration. Hybrid chemical-electrochemical flow-assisted processes have been developed to provide a homogeneous surface finishing in parts with complex shape, like 3D lattice structures. While such techniques are now successfully applied to medical-grade alloys, they have not been established for single-phase materials, like CP-Ti and tantalum, which exhibit higher corrosive resistance. Still, single-phase metals offer several key engineering and biological advantages, making them attractive for applications with lower load-bearing requirements, such as spinal fusion, and oral and maxillofacial surgery. Hence, the final part of this thesis (**Chapter 8**) aims to investigate the feasibility of applying chemical-electrochemical surface treatment on CP-Ti lattice structures to improve fatigue strength and enhance osteoconduction.

Ultimately, all the results presented in this thesis should collectively disclose a novel perception for designing, manufacturing and post-processing lattice structures for next-generation bone replacement implants. Better orthopaedic implants should integrate lattice structures with bone-matching mechanical properties across different scales, topologies that enhance bone tissue formation, and surfaces that provide bioactivity.

4. Materials and methods

4.1. Lattice structures design process

This work employed strut-based lattice structures of either stochastic or periodic micro-architecture which were designed using Rhinoceros 3D modelling software (Robert McNeel & Associates, USA). All structures were represented as networks of zero-thickness lines as described in (89). This is a convenient way of designing complex lattice structures as it is computationally efficient and allows high control during the additive manufacturing process as explained later.

4.1.1. Stochastic structures

The stochastic structures were designed by filling a design volume (corresponding to the shape of a part) with pseudo-randomly distributed points. The points (nodes) were then connected with lines (i.e., the struts) to achieve a desired nodal connectivity Z (i.e., the number of lines connected to each node).

An arbitrary design parameter, termed as strut density d (struts per mm^3), was used to estimate the initial number of needed points. Using part's volume V and strut density d , the number of struts s can be expressed as:

$$s = d \cdot V \quad \text{Equation 4.1}$$

Hence, for a given connectivity Z , the required nodes j can be approximated based on (112) as:

$$j \approx \frac{2 \cdot s}{Z} \rightarrow j \approx 2 \cdot V \cdot \frac{d}{Z} \quad \text{Equation 4.2}$$

Nodes with $Z=1$ with floating struts – typically introduced at the outer surface of a part – were removed since they do not contribute to the structure's mechanical response and significantly lower the average connectivity of the structure.

Structures' points were generated using the embedded feature "*3D populate*" in Rhinoceros by inputting the desired number of points (close to the ideal j), the part geometry and a seed number (an integer to initiate the pseudo-random numbers generation). This tool iteratively generates points: in each iteration, a small number of points is spatially generated and the point furthest from all existing points is added to the final point set.

This method was chosen for its efficacy in generating stochastic topologies regardless of the part's shape, leading to highly isotropic structures. The final structures consist of lines in all directions, and the struts have the same angle distribution regardless of connectivity Z . This is advantageous from a design perspective, as it allows better prediction of lattice behaviour when integrated into an implant. Other common methods for generating stochastic structures include Voronoi decomposition and Delaunay triangulation (91). Regardless of the method used, it is important to note that the final structures are pseudo-random, meaning they can be identically reproduced with the same input parameters, enhancing lattice behaviour predictability.

4.1.2. Periodic structures

Periodic structures were also represented as line networks by spatially stacking a unit cell type. Periodic structures in this work were used as mechanical testing coupons, hence the unit cells were represented as prisms with edge lengths l_x , l_y and l_z in respect to the coordinate system.

Equations 4.1 and 4.2 can also be applied to periodic structures using the strut number s , nodal connectivity Z and volume of the unit cell to calculate the corresponding strut density. This was useful for correlating strut density and relative density similarly to stochastic lattices.

4.1.3. Printing geometries preparation

In powder bed fusion systems, particularly in the selective laser melting method utilised in this work, the fabrication of low-angled overhanging surfaces, such as struts, with respect to the printing bed is challenging. This difficulty arises because successive layers often have smaller overlaps, resulting in inadequate support along the strut. Minimum printable inclination angles have been reported to range from 10° to 45° (115,318,319), depending on powder material, laser parameters and strut length.

In the setup employed for this work, a limit of 25° was found to ensure high reproducibility of struts of varying lengths. To avoid horizontal and low-angle struts, lines with an inclination angle $<25^\circ$ were kinked by converting the sloping lines into sloping isosceles triangles, with equal angles set at 25° (115).

Vertical supporting struts were placed at the bottom surface of specimens to ensure that their actual height closely matched the nominal value after removal from the substrate plate.

The final line geometries were sliced using in-house software which assigns laser parameters to each line depending on their inclination angle with respect to the building bed as described in (48). This allows for the fabrication of structures with a uniform strut thickness t . The design and manufacturing workflow is depicted in **Figure 4.1**.

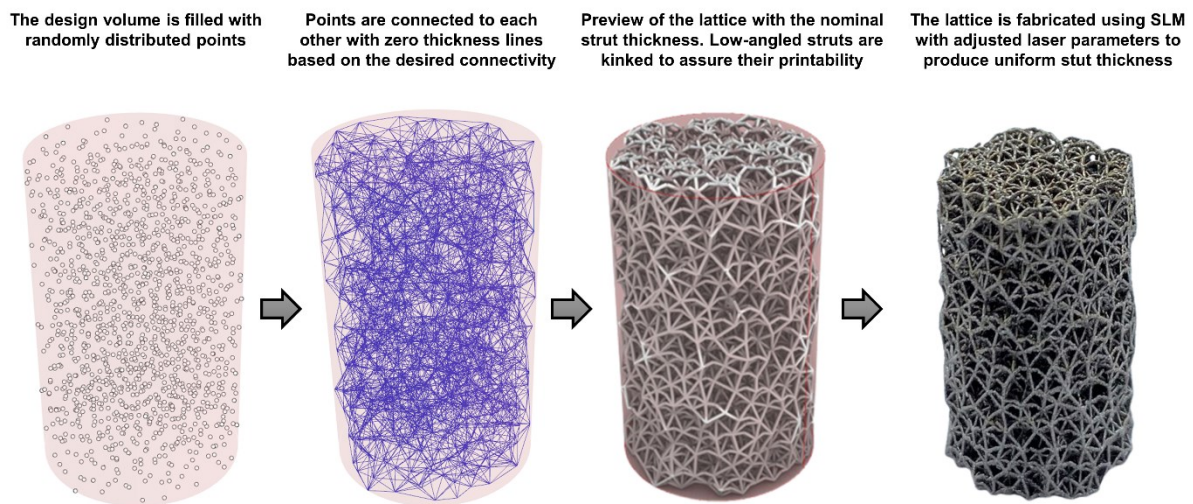


Figure 4.1. Workflow for the generation of stochastic lattice structures. Example of a stochastic specimen with connectivity $Z=12$, strut density $d=5$, and strut thickness $t=230 \mu\text{m}$. Figure reproduced under CC BY license from (109).

4.2. Additive manufacturing and specimen preparation

Lattice structures were fabricated using an AM250 metal powder bed fusion system (Renishaw plc., UK) and commercially pure titanium Grade 2 powder (Carpenter Additive, USA) with a particle size range of 10–45 μm (D50: 27 μm) (**Figure 4.2A**).

A contour laser scanning strategy was used with 70 μm beam diameter, 50 μm hatching spacing, and 50 μm layer thickness. Consecutive laser firing point distance δ_p was also fixed at 50 μm . Hence, strut thickness was controlled by varying laser power P [W] and scan speed through exposure time δt [μs]. For this purpose, linear regression models connecting the specific enthalpy ΔH [J/kg] – using *equation 2.11* – and nominal strut thickness t were developed for 5 ranges of strut inclination angle (**Figure A.1** in **Appendix**) as per (89). Laser parameters are depicted in **Figure 2.7**.

Five nominal strut thickness values – 170 μm , 230 μm , 260 μm , 300 μm and 380 μm – were assessed to develop the regression models. This range was chosen to align with human trabeculae thickness (150 to 300 μm) (36) while allowing for extrapolation to larger struts. However, 170 μm was the thinnest strut that could be consistently fabricated with the given printer and powder. The corresponding laser parameters are detailed in **Table A.1** in the **Appendix**.

After printing, the oxidised powder accumulated around the edges of the build (substrate) plate was discarded, while the remaining powder covering the build plate was collected for sieving and reuse in subsequent builds. A sieve with mesh openings of ≤ 45 μm , compliant with ASTM B214–99, was used to ensure that the particle size of the recycled powder remained within the specified range.

Once the build plate was removed from the machine, compressed air was applied to dislodge excess powder trapped within the lattice specimens. Specimens were removed from the substrate using wire electro-discharge machining (AQ400L, Sodick Inc., Japan) using water as the dielectric fluid, 30 V and a feed rate of 0.4 mm/min. They were then ground parallel to trim any supporting strut left and cleaned ultrasonically in acetone or distilled water.

Finally, specimens were weighted with an analytical scale and measured with micro-callipers to estimate their relative density. The apparent density ρ of each specimen was taken equal to the weight divided by the measured volume, while the density of solid CP titanium at room temperature ρ_s was taken equal to 4.51 g/cm³ (320). Gravimetric porosity was calculated using *Equation 2.1*.

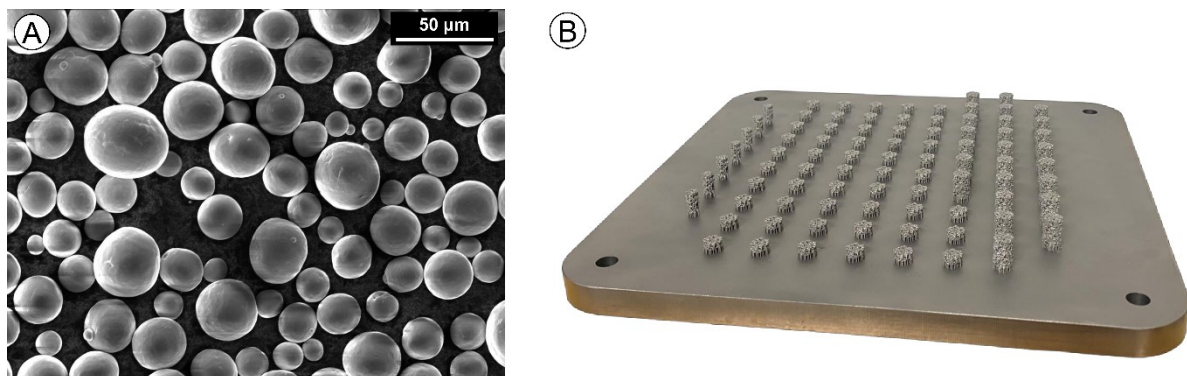


Figure 4.2. Representative images of the powder feedstock and a build plate. (A) SEM image showing the spherical CP-Ti powder. The powder was reused between builds; thus, particles demonstrated rough surfaces and aggregations. (B) Lattice structure coupons were printed vertically with supports only at the bottom surface.

4.3. Post-processing

Stochastic lattice specimens with $Z=8$ and $d=8$ struts/mm³ were fabricated using a nominal strut thickness of 300 μm . Specimens were cylindrical with two sets of dimensions: $\text{Ø}8 \times 12$ mm (used for mechanical testing) and $\text{Ø}8 \times 3$ mm (used for cell culture).

Specimens were left attached to the build plate (**Figure 4.2b**) and were post-processed using a chemical–electrochemical surface treatment (Hirtisation®, RENA Technologies, Austria) using an H3000 automated industrial finishing machine. The system operates at 25°C for 4 min using the electrolyte Hirtisation® Ti-Auxilex, comprising a mixture of methanesulfonic acid, $\text{CH}_4\text{O}_3\text{S}$, and phosphonic acid, H_3PO_3 specifically designed for Ti alloys. After treatment, the plate was rinsed with distilled water and vacuum dried at 50°C. Specimens were removed from the plate using EDM, rinsed with distilled water and air-dried.

The strut thickness and roughness reduction were evaluated using micro-CT scanning as detailed below. Then, new lattice specimens of the same design were fabricated using the strut thickness found in treated specimens. This was done to compare the treated lattices with as-built equivalents of the same strut thickness.

This was part of the final chapter (**Study V**), and design parameters were selected so that the post-processed lattice would employ beneficial characteristics according to mechanical and biological results from the preceding studies.

4.4. Mechanical characterisation

4.4.1 Quasi-static compression

Quasi-static compression testing was performed following ISO 13314:2011 (321) using universal testing machines (Instron 5565 or Instron 8874) with a 5 kN, 10 kN or 25 kN load cell depending on the specimen. Specimens were centred and compressed between two parallel hardened ($>62\text{HRC}$) lubricated platens in position control. Two linearly variable differential transformers (D6/05000A, RDP Electronics Ltd, UK) measured the displacement. All specimens were measured before testing with a calliper. Compression was performed under a constant strain rate of 0.0016 s^{-1} ; hence displacement rate was adjusted to each specimen's height. Stress-strain curves were generated using the engineering stress and strain.

For each specimen design, a pilot specimen was compressed to 40% strain to estimate the post-yield plateau stress σ_{pl} (average recorded stress between 20% and 30% strain). Five specimens per combination were subsequently compressed to 35% with a hysteresis loop before yield starting at 70% and reversing to 20% of σ_{pl} . Using the acquired stress-strain curves, the elastic modulus E (assumed equivalent to structure's stiffness) was calculated as the slope of the hysteresis loop's linear regression. Yield stress was estimated using the 0.2% plastic strain offset. Depending on the stress-strain curve shape (see representative examples in **Figure 4.3**), the ultimate compressive strength σ_u was taken as the first (local) maximum stress or, in case of no distinguishable local maximum, the stress at 1% plastic strain offset from the hysteresis loop. Last, energy absorption was calculated as the area under the stress-strain curve up to 30% strain, excluding the hysteresis loop. Energy absorption was only calculated for bend-dominated lattices as only these are used for energy absorption applications since they do not exhibit stress softening or shear band fracture after yield.

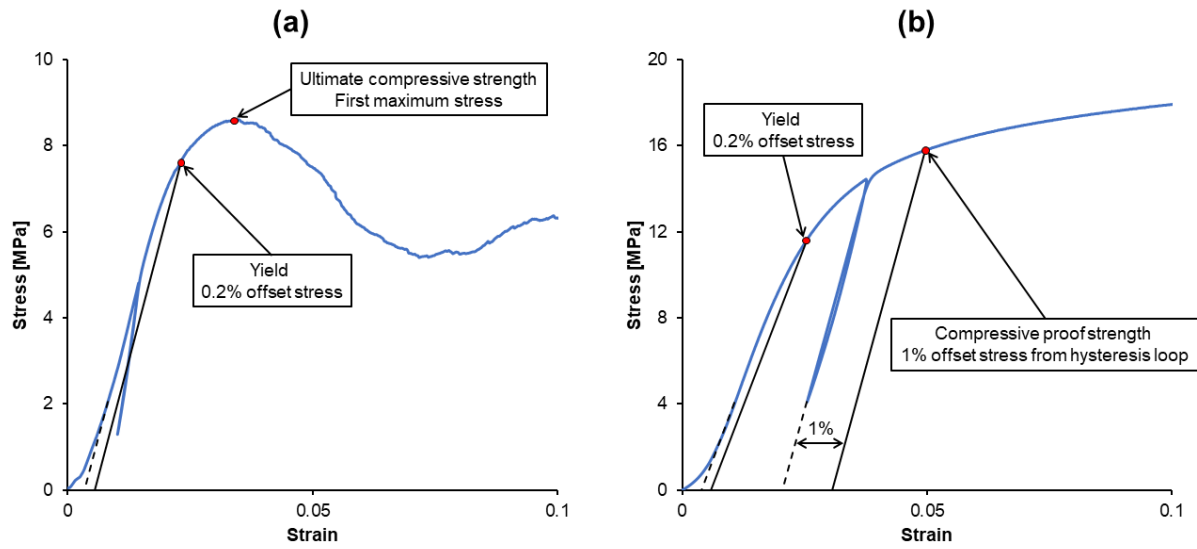


Figure 4.3. Representative compression stress-strain curves. Depending on lattice type, specimens can exhibit (a) local maximum stress or (b) post-yielding plateau stress. Figure reproduced under CC BY license from (322).

Study I – The effect of design parameters of stochastic lattices on mechanical behaviour

To investigate the effect of design parameters on the mechanical properties of stochastic lattices, twelve lattice designs were generated using different combinations of connectivity Z and strut density d . Specimens were cylindrical, $\varnothing 13 \times 21$ mm, and the values of the design parameters, the Maxwell number M (Equation 2.4) and the strut length of each design are provided in **Table 4.1**.

6 replicates were fabricated for each stochastic design using two nominal strut thickness values (230 and 300 μm). Hence, a total of 144 specimens were tested in quasi-static compression. Examples of fabricated specimens are shown in **Figure 4.4**.

Before testing, the relative density for each design was calculated and power law models (Equation 2.3) were used to correlate the strut thickness-to-length ratio to relative density. This analysis was used to investigate whether connectivity affected relative density.

Table 4.1. Design parameters of tested stochastic structures			
Average nodal connectivity Z	Strut density d [struts/mm ³]	Maxwell number M	Strut length L [μm] *
4.0	3.0	-4291	910 ± 200
4.0	5.0	-6713	770 ± 160
4.1	7.0	-8908	690 ± 140
8.3	3.0	2302	1260 ± 200
8.3	5.0	3898	1060 ± 170
8.3	7.0	5433	940 ± 150
12.0	3.0	4212	1580 ± 310
12.1	5.0	7043	1330 ± 260
12.2	7.0	9891	1190 ± 230
13.9	3.0	4726	1740 ± 360
14.0	5.0	7994	1450 ± 300
14.1	7.0	11248	1300 ± 260

* Values rounded to nearest 10.

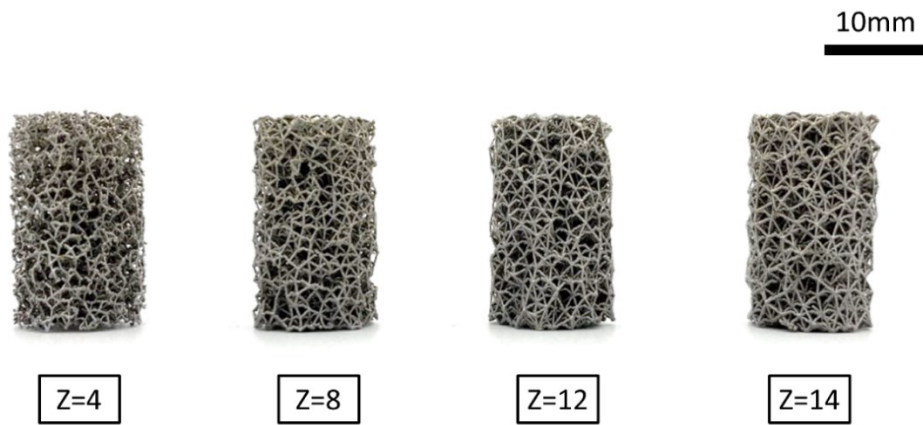


Figure 4.4. Stochastic lattice specimens with different connectivity levels Z . All imaged specimens had strut density $d=3$ and strut thickness $t=230$ μm. Figure reproduced under CC BY license from (109).

Study II – The effect of lattice dimensions on mechanical properties

Four lattice topologies displaying either bend-dominated or stretch-dominated micro-deformation modes were included in this study. The selected topologies (**Figure 4.5**) were:

- A high-connectivity ($Z = 8$) stochastic lattice, being stretch-dominated ($M > 0$, **Table 4.1**)
- A low-connectivity ($Z = 4$) stochastic lattice, being bend-dominated ($M < 0$, **Table 4.1**)
- A body-centred cubic (BCC), being bend-dominated (319)
- An octet-truss, being stretch-dominated (323)

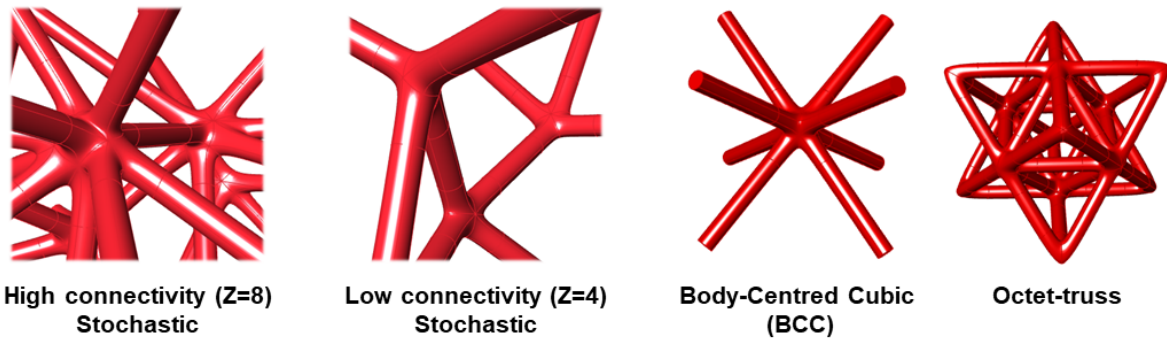


Figure 4.5. Four lattice topologies were tested using different aspect ratios.

In the first part of the study, specimens were designed large enough (composed of more than 7 unit cells or pores along their edge lengths to isolate the effect of aspect ratio without the influence of size-effects (133,135,136). For this purpose, specimens were designed with 7 aspect ratios of comparable volumes as shown in **Table 4.2**. Stochastic structures were cylindrical, while the periodic lattices were prisms. In both periodic lattices, the unit cells were cubic with edge length l equal to 1, 1.5 or 2 mm for the BCC, and 1.3 mm for the octet-truss. A minimum of 13 and 10 unit cells were used to form the edges of the BCC and octet-truss lattices, respectively.

Testing was structured to first identify the effect of aspect ratio on the stretch-dominated stochastic and bend-dominated BCC structures. Then, a reduced set of compression tests was performed on the stretch-dominated octet-truss and the bend-dominated stochastic structures to confirm the results were not limited to specific lattice topologies. Last, two extra bend-dominated BCC structures with lower relative density and lower slenderness ratio (strut thickness-to-length ratio t/L) to investigate whether the effect of aspect ratio is independent of relative density.

Table 4.2. Nominal dimensions of large-size specimens with different aspect ratios						
Aspect ratio ζ	Height to diameter/width ratio (H/D or H/W) [mm/mm]					
	BCC ($l=1$ mm)	BCC ($l=1.5$ mm)	BCC ($l=2$ mm)	Stochastic Z4	Stochastic Z8	Octet-truss ($l=1.3$ mm)
0.50	13/26	19.5/39	26/52	13/26	13/26	13/26
0.65	13/20	-	-	-	13/20	-
1.00	13/13	-	-	-	13/13	-
1.60 or 1.62	21/13	31.5/19.5	32/20	21/13	21/13	20.8/13
2.00	26/13	-	-	-	26/13	-
2.54	33/13	-	-	-	33/13	-
3.00	39/13	58.5/19.5	60/20	39/13	39/13	39/13

In the second part of the study, specimens were designed with small dimensions to encounter size effects. Only BCC and octet-truss lattices were included to allow high control over the arrangement of unit cells along specimen edges. Specimens were designed with 3 aspect ratios: low ($\zeta=0.5$), intermediate ($1<\zeta<2$), and high ($\zeta=3$). To introduce the size effects, the shortest edge for each aspect ratio was represented with either 3 or 5 unit cells as described in **Table 4.3** and depicted in **Figure 4.6**.

Table 4.3. Nominal dimensions of small-size specimens with different aspect ratios			
Aspect ratio ζ	Unit cells along the smallest edge	Height to width ratio (H/W) [mm/mm]	
		BCC ($l=1$ mm)	Octet-truss ($l=1.3$ mm)
0.50	3	3/6	3.9/7.8
	5	5/10	6.5/13
1.60 or 1.67	3	5/3	6.5/3.9
	5	8/5	10.4/6.5
3.00	3	9/3	11.7/7.8
	5	15/5	19.5/6.5

A total of 156 large-sized specimens and 72 small-sized specimens (6 replicates per specimen design) were fabricated. Specimens of the same lattice type were printed in the same batches so that they would not be affected by the degree of powder recycling. All specimens were printed vertically with supports only at the bottom surface to eliminate the effect of printing angle (196) when comparing specimens of the same type.

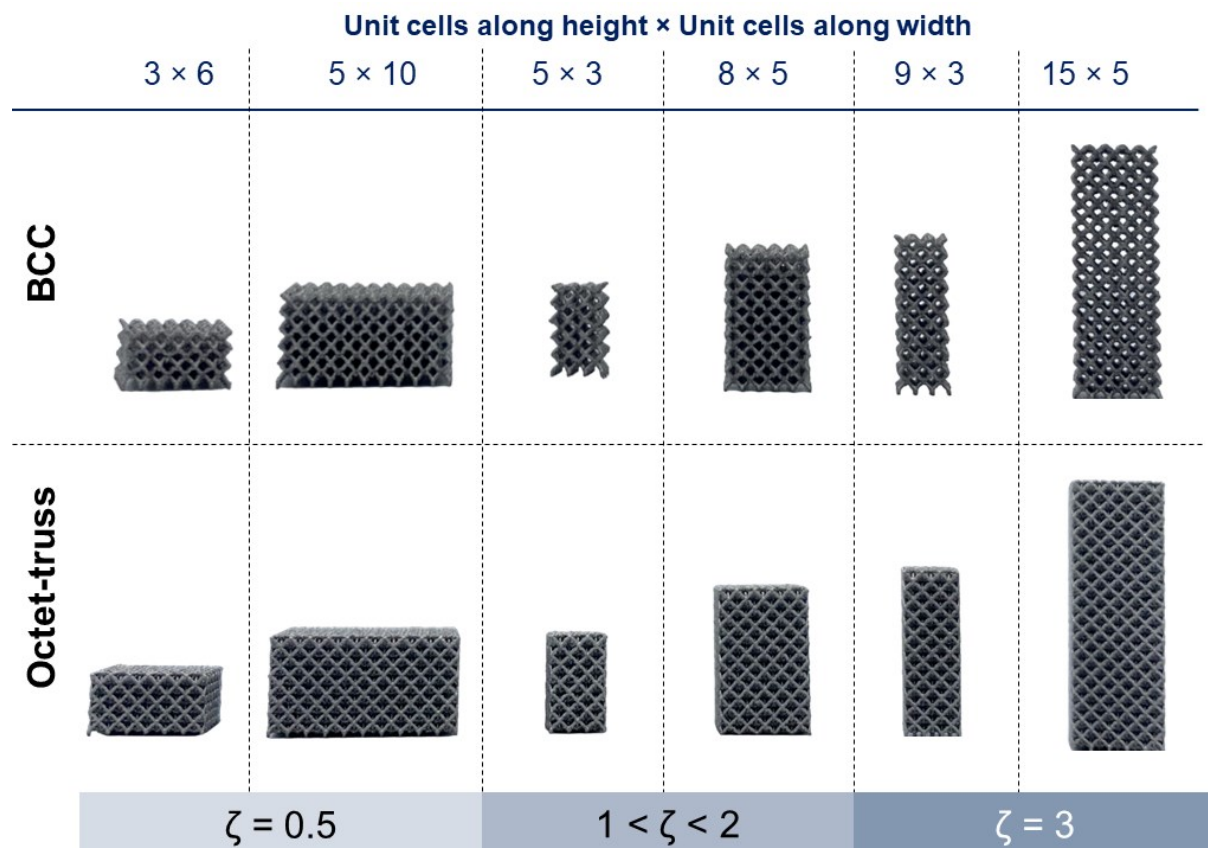


Figure 4.6. Small-sized periodic lattice specimens with different aspect ratios.

Study V – The effect of surface treatment on CP-Ti lattices

This study aimed to compare the quasi-static properties of surface treated specimens with untreated (as-built) conditions. The as-built specimens employed the same lattice design but with (a) 300- μm struts (i.e., thickness before treatment) and (b) equal-sized struts as the treated structures. All specimens were cylindrical ($\text{Ø}8 \times 12 \text{ mm}$) with stochastic architecture (Z8-d8).

4.4.2. Digital image correlation analysis

Digital image correlation (DIC) was used to measure the full-field planar strain distribution within lattice structures during quasi-static compression testing. A digital camera (1080p resolution, 30 fps, 750D Canon, Japan) positioned normally to the lattice structures recorded the loading operation from intact to deformed states. Each video was post-processed in 2-dimensional DIC software (GOM Correlate Professional, Germany). A facet size of 19 pixels and 50% overlap was used throughout to track the visible strut features of the lattice structures. The field of view was adjusted so that strut features had a size of 3-5 pixels, to minimise aliasing and degrading spatial resolution during compression testing. The (%) longitudinal strain ε_z defined as the strain in the loading direction was used to assess the influence of topology and aspect ratio of the lattice structures (**Study II**).

4.4.3. Cyclic compression

Uniaxial compression-compression testing was performed in accordance with ISO 1099:2017 (324) using a universal testing machine (Instron 8872 or Instron 8874) with a 1 kN or 10 kN load cell depending on the applied forces. Specimens were tested in load control at a frequency of 15Hz (sinusoidal waveform) with $R=0.1$. The number of cycles at 5% strain was taken as the fatigue life.

Study I – The effect of design parameters of stochastic lattices on mechanical behaviour

Fatigue testing was used to assess the effect of connectivity in stochastic lattice structures. Specimens ($\varnothing 13 \times 21$ mm) were designed with four connectivity levels ($Z = 4, 8, 12, 14$), but fixed strut density ($d=5$) and strut thickness ($t=230$ μm). An additional design with $Z=4$, $d=5$ and $t=300$ μm was tested to isolate the effect of connectivity Z while maintaining the same relative density (21%) as the specimen with $Z=14$, $d=5$ and $t=230$ μm .

Two specimens per combination were tested at five stress levels (80%, 65%, 50%, 35%, and 25% of σ_u) to produce the S-N curves. If the fatigue life at a stress level demonstrated >40% difference between the two measurements a third measurement was taken as proposed in (171,172). The modified staircase method (ISO 12107:2012) (325) was used to find the fatigue life at 10^6 cycles with an initial stress of 20% σ_u and step size of 1.25% σ_u using 6 specimens for each combination of design parameters (230).

Tests were terminated after 1 million cycles or when the moving platen reached 10% compressive strain. A total of 83 specimens were used for this study, all printed within the same batch.

Study V – The effect of surface treatment on CP-Ti lattices

The same specimen conditions used in the quasi-static compression tests were evaluated in fatigue. For clarity, these were: the treated, the before-treatment (300 μm struts), and the as-built equivalent.

Two specimens ($\varnothing 8 \times 12$ mm) per condition were tested at five stress levels (80%, 60%, 50%, and 30% of the quasi-static compressive strength). All tests were terminated either when 10% compressive strain was reached (i.e., ensuring catastrophic failure) or when a specimen survived 2 million cycles.

4.5. *In vitro* cell cultures

Static *in vitro* cultures were conducted by seeding cells on stochastic lattice structures to evaluate the effect of topology and surface modifications on major cell activities: adhesion, viability and proliferation, ECM formation, and differentiation.

First, a pre-osteoblast cell line was used to assess the effect of lattice design parameters (connectivity, strut density, and strut thickness). As a next step, human mesenchymal stromal cells (hMSC) were used to assess the effect of surface modifications from the initial adhesion of primary cells until osteogenic differentiation. The surface modifications were aimed at enhancing the biocompatibility of titanium scaffolds, without carrying chemical molecules to provide osteogenic signalling. Hence, in the case of hMSCs, bone-specific growth factors were supplemented through the culture media to provide stimulation towards osteogenic differentiation.

All cells were purchased from accredited suppliers. They were first expanded in flasks and were proliferated for up to 4-5 passages until a desired number of cells was reached. Passages were performed at 70% or 80% confluency and Trypsin-EDTA solution (25200072, Gibco™, USA) was used for cell dissociation. After each passage and before cell seeding, cells were counted using a Neubauer chamber. All cultures were performed under standard culture conditions (37°C and 5% CO₂ in a humidified environment). Hereafter, in the context of biological experiments, lattice structures will be referred to as scaffolds.

4.5.1. Pre-osteoblast culture

MC3T3-E1 Subclone 4 mouse pre-osteoblasts (CRL-2593, American Type Culture Collection, USA) were used to assess the effect of design parameters of stochastic titanium scaffolds. **Study IV** focused on assessing the effect of connectivity and strut density while keeping fixed strut thickness. **Study V** aimed to compare scaffolds with low and high strut thickness while designed with the same Z and d .

A different batch of cells was used for each study. In both cases, cells were expanded with a complete culture medium containing MEM (11095080, Gibco™, USA) supplemented with 10% FBS (A5256801, Gibco™, USA) and 1% antimicrobial solution (Penicillin-Streptomycin, 15140122, Gibco™, USA). The same medium was used for the 3D cell cultures in scaffolds.

Study III – The effect of connectivity and strut density within stochastic lattices

Specimens for this study were designed as cylinders with dimensions Ø12×6 mm. Four stochastic scaffold designs were created using two connectivity levels ($Z=4$ and $Z=8$) and two values of strut density ($d=3$ and $d=7$ struts/mm³). For convenience, they will be referred to as Z4-d3, Z4-d7, Z8-d3, and Z8-d7.

Scaffolds were cleaned ultrasonically in acetone, left immersed in 4M NaOH for 24 h and then washed with phosphate-buffered saline (PBS) to stabilise their pH value to 7.4. NaOH was used to remove any contaminants in scaffolds and increase titanium surface hydrophilicity (299). The difference in the surface topography due to alkali treatment with NaOH is shown in **Figure A.4** in the **Appendix**.

Prior to cell seeding, scaffolds were autoclaved, then immersed in culture medium and incubated for at least 2 h to allow for surface protein adsorption (326). Pre-wetted scaffolds were placed in 24-well plates for cell suspension. A total of 1×10^6 cells/scaffold were suspended on the scaffolds, half on the top and half on the bottom surface. After cell suspension on each surface, scaffolds were incubated for 1 h to allow cell attachment within the porous structures. Seeded scaffolds were then transferred to 12-well plates and filled with 5 mL of complete culture medium. Cell-seeded scaffolds were cultured for a total of 28 days. Media changes were performed every 3 or 7 days as needed.

Study IV – The effect of strut thickness within stochastic lattices

Specimens for this study were designed as cubes with 6 mm edge lengths. One scaffold design was used with $Z=6$ and $d=3$ struts/mm³. Scaffolds were fabricated using two extreme strut thickness sizes: 170 µm and 300 µm.

Scaffolds were cleaned ultrasonically in acetone without any further processing. Following autoclave and pre-wetting, they were placed in 24-well plates and seeded with 5×10^5 cells/scaffold. After allowing 1 h for cell attachment, they were transferred to new 24-well plates and filled with 2 mL of complete culture medium. In this case, cell-seeded scaffolds were cultured for a total of 21 days and media changes were performed every 2 or 3 days.

4.5.2. Mesenchymal stromal cell culture

Human mesenchymal stromal cells derived from bone marrow (SC00BM1, Vitro Biopharma, USA) were used to assess the effect of surface treatment. Cells were first expanded with a complete culture medium containing MEM-alpha (22571020, Gibco™, USA) supplemented with 10% FBS and 1% antimicrobial solution.

All scaffolds ($\varnothing 8 \times 3$ mm) for this study were rinsed with distilled water, air-dried and autoclaved before cell seeding. Scaffolds were seeded without a pre-wetting step to bring out any effect from the surface treatment on cell adhesion. A total of 75,000 cells/scaffold were suspended on scaffolds using the complete medium and left in the incubator for 1h to allow cell attachment. The cell-seeded scaffolds were then transferred to 24-well plates and filled with 1.5 mL of complete culture medium. After 24h, cells were transferred to new 24-well plates to continue the cell culture only with the presence of cells attached to the scaffolds. For the first 3 days, the complete medium was supplemented with 10 ng/mL FGF-2 (PeproTech Group, UK) to promote cell proliferation. On the third day, the same medium was supplemented with 10 mM β -glycerophosphate (50020, Merck, USA), 100 nM dexamethasone (D4902, Merck, USA), and 20 ng/mL IGF-1 (PeproTech Group, UK) to promote osteogenic differentiation. Media changes were performed every 3 or 4 days.

4.6. Biological assays

4.6.1 Assessment of cell adhesion and cell proliferation

A high-resolution cell viability kit (CCK-8, ALX-850-039, Enzo Life Sciences, USA) was used to assess cell adhesion within all scaffold designs, 24h after seeding. Cell-seeded scaffolds were transferred to 24-well plates and incubated for 4 h with 0.8 mL of fresh culture medium and 80 μ L of CCK-8 solution. Stained media were then added to a 96-well plate and the absorbance was measured at 450 nm using a microplate reader (CLARIOstar Plus, BMG LABTECH, Germany). The assay was also performed using the initial well plates (where the same scaffolds had been seeded) to account for the cells that did not adhere to the scaffolds to estimate cell seeding efficiency. This assay was used only in **Study III**.

Cell viability and proliferation rate on scaffolds were evaluated by measuring cellular metabolic activity up to the first 10 days using a resazurin-based assay. For that purpose, 10% v/v AlamarBlue® (DAL1100, Invitrogen, USA) was added to each well of all scaffold designs and incubated for 4h. Stained media from the wells were replaced with fresh culture media to continue their culture, given that resazurin is a non-toxic reagent for cells. Stain media were used to measure fluorescent intensity at 560 nm excitation and 600 nm emission. This assay was used in all cell cultures and measurements were taken every 3 days.

4.6.2. Quantification of collagen content

Newly formed ECM was evaluated for collagen concentration at days 7 and 28 using Picrosirius Red staining (1 mg/mL Sirius Red in saturated Picric acid). Scaffolds were initially stored in 10% neutral buffered formalin, cleaned with PBS, and immersed in Picrosirius Red overnight. All scaffolds were then washed with distilled water to remove any unbound dye and dried at 37°C. Collagen content was quantified using colourimetry. Bounded dye was removed by immersing scaffolds in 0.1 mol/L NaOH in PBS and incubating for 45 min with 30 rpm shaking. Afterwards, the absorbance of the dissolved dye was measured at 550 nm using a microplate reader. This assay was used only in **Study III**.

4.6.3. Cell differentiation

The progress of cell differentiation was assessed by measuring the expression levels of key biomarkers that are linked to the early and late stages of osteogenesis. A variety of methods was used for this purpose in each study as detailed in this section. It should be noted that in **studies III** and **IV** a pre-osteoblast cell line, these cells are committed to osteoblastic differentiation, hence a more appropriate term to characterise their functionality state would be “maturation” rather than “differentiation”. The term differentiation will be used only in the case of **Studies V** which employed hMSCs.

Pre-osteoblast maturation – Study III

Alkaline phosphatase activity was measured through supernatants collected from scaffolds on days 7, 14, 21 and 28 using a fluorometric ALP assay kit (MAK411, Sigma-Aldrich, USA). This method allowed monitoring of the ALP expression within the same samples throughout the culture. The kit calculates the amount of the enzyme that hydrolyses 1 μ mol of the reagent per minute [μ mol/min/mL]. This measurement may be proportional to the number of cells. Since values were not normalised to cell number, data analysis was focused on assessing the progress of ALP activity within the same scaffold design without directly comparing different designs.

Additionally, the expression levels of collagen type-I, osteopontin and osteocalcin were measured on days 7 and 28 through quantitative real-time Polymerase Chain Reaction (RT-qPCR). For the RT-qPCR analysis, cells were extracted from scaffolds using Trypsin-EDTA solution and stored in RNeasyTM Stabilization Solution (AM7021, Thermofisher, USA). Nucleic acid extraction was performed using the RNeasy kit (74104, QIAGEN, Germany) and the GoTaq 1-step RT-qPCR kit (A6020, Promega UK Ltd, UK) was used for the analysis. The PCR reaction methodology is described in detail in the **Appendix**. The sequences of the selected qPCR primers are shown in **Table 4.4**. The relative expression levels of the genes of interest compared to untreated samples were determined and normalised to the expression of the reference gene (β -actin) using a modified $\Delta\Delta C_t$ method.

Table 4.4. PCR primer sequences for multiple RNA targets in mouse			
Target	Forward 5' → 3'	Reverse 5' → 3'	Ref.
Collagen Type-I	CCTCAGGGTATTGCTGGACAAC	CAGAAGGACCTTGTTTGCCAGG	(327)
Osteopontin	CCTCCCGGTGAAAGTGAC	CTGTGGCGCAAGGAGATT	(328)
Osteocalcin	TGCTTGTGACGAGCTATCAR	GAGGACAGGGAGGATCAAGT	(329)
β-actin	GGGTCAGAAGGACTCCTATG	GGTCTCAAACATGATCTGGG	(328)

Pre-osteoblast maturation – Study IV

At days 1, 7 and 14, scaffolds (n=3 per type and day) were washed with PBS and immersed in an Eppendorf tube containing distilled water with 0.2% Triton™ X100 for 20 min followed by 10 min ultrasonic treatment and 10 min centrifugation at 16,000×g at 4°C. Intracellular ALP activity was measured using the same fluorometric ALP assay kit through cell lysates. Additionally, the total DNA content in cell lysates was measured using the Quant-iT™ dsDNA Assay Kit (Invitrogen, USA). This measurement provides the mass [ng] of DNA in the cell lysate solution [μL]. ALP activity was normalised to DNA content and expressed as μmol/min/μg of DNA (330).

Using cell lysates from day 14 (n=3 per type), an ELISA kit was used to determine the osteocalcin content (A3289, Antibodies.com, UK). The kit employed a pre-coated 96-well microtiter plate, and the concentration of osteocalcin was calculated by measuring the absorbance at 450 nm. Osteocalcin content was normalised to DNA content and expressed as pg of osteocalcin per μg of DNA.

MSC osteogenic differentiation – Study V

To assess the progress of osteogenic differentiation, the alkaline phosphatase activity in the supernatants collected on days 7, 13, and 17 was measured using a fluorometric ALP assay kit (ab83371, Abcam, UK). Additionally, the total protein content in the supernatants [μg/mL] was measured using the Pierce™ BCA Protein Assay kit (10678484, Fisher Scientific Ltd., UK). ALP activity was normalised to protein content and expressed as μmol/h/μg.

4.7. Imaging and image-processing

4.7.1. Micro-CT scanning and lattice structure characterisation

Lattice specimens were μCT-scanned with an Xradia 510 Versa (Carl Zeiss AG, Germany). Scans were performed at 80 kV using 2401 projections, 5 sec exposure time and a voxel size ranging from 10 to 20 μm depending on specimen size. Scans were reconstructed using the Scout-and-Scan software (Carl Zeiss AG, Germany). Reconstructed images were converted into binary images by applying the IsoData threshold algorithm (331) using the open-source Fiji image-processing software (National Institutes of Health, USA).

Lattice structure morphological characterisation

CTAn (Bruker Ltd., United Kingdom) was used to quantify the (open) porosity, surface area to structure's volume ratio and the enclosed porosity of imaged structures. In addition, trabecular separation was used as an estimate of the mean pore size in lattice structures. While related, these two metrics are distinct. Trabecular separation is traditionally used to describe the average distance between adjacent trabeculae in cancellous bone. It is calculated by fitting the largest possible sphere at each voxel within the void space constrained by the nearest trabeculae or struts, determining its diameter, and averaging these values across the entire void volume (332). In contrast, pore size is typically measured by identifying discrete pores and calculating the diameter of the largest inscribed sphere that fits within each pore. In the context of lattice structures in this work, trabecular separation provides a spatially averaged measure of strut distance. For validation, strut distance (measured through micro-CT scans) was compared to the theoretical pore sizes (measured geometrically) within the periodic lattice structures as described in the **Appendix**.

Strut morphological evaluation

The open-source software StrutSurf (333) was used to assess the thickness and surface roughness of struts. Briefly, using the binary images, the software creates 3D representations of sub-volumes inside the structure and allows a cylindrical mask to be placed around individual struts. The axis of the cylinder in respect to the scan plane is being calculated and strut thickness t is taken as the diameter of the mean circle that fits the object's projections. Using the 3D representation of the strut, the (arithmetic average) surface roughness Sa and the maximum peak-to-valley surface roughness Sz – calculated as defined in ISO 25178-2 (334). For this purpose, a sample of 20 struts of different build angles in each specimen was measured.

4.7.2. 3D Optical Profilometer

Individual struts were printed at different angles with respect to the building bed and their surface roughness was measured using a digital microscope (RH200, Hirox, Japan). For this purpose, the heights along a 75- μm long profile line were acquired using a 0.55 μm linear step, 0.39 μm resolution and $\times 400$ dry objective lens. Three unique struts were used per building angle and the arithmetic average roughness (Ra) for two profiles per strut (one at the upward and downward-facing surface as printed) was calculated as per ISO 4287 (335). This technique compared the roughness between the upward and downward-facing strut surfaces and was employed in **Study III**.

4.7.3. Scanning electron microscopy (SEM)

Plain structures

Scanning electron microscopy (Mira, TESCAN, Czech Republic) was used to observe and evaluate the quality of the additively manufactured specimens. Images were taken using 5 keV and 1 nA.

Structures containing organic matter

For specimens used in cell culture, SEM was used to observe the morphology of cells and formed ECM at different time points during the culture. Cell-seeded scaffolds were fixed in 10% neutral buffered formalin, then cleaned with PBS, dehydrated through ethanol series (30%, 50%, 70%, 96% and 100% ethanol dilution) and air-dried for 24 h. Scaffolds were sputter-coated by a 15-nm-thick chromium layer and imaged at 10 keV and 1 nA.

4.7.4. Fluorescence microscopy

Cell Viability

A viability/cytotoxicity (live/dead) assay kit (30002, Biotium, USA) was used to stain live and dead cells at different time points during the culture. One sample per condition and day was used for the assessment. Samples were washed with PBS twice and then incubated for 45 min in PBS containing Calcein AM and Ethidium Homodimer III dyes for staining live and dead cells, respectively. Samples were then removed from the well plates and imaged in an SP8 confocal microscope (Leica, Germany) according to the manufacturer's instructions.

Mineralised matrix labelling

Fluorochrome dyes were added to culture media to assess mineralised ECM regions during the culture. Scaffolds were incubated for 24 h, washed with PBS, fixed in 10% neutral buffered formalin, cleaned with PBS, and air-dried. Scaffolds were imaged in a Thunder widefield microscope (Leica, Germany), and the acquired images were post-processed using the microscope's software. The following dyes, known to bind to calcium crystals, were used (336):

- Calcein (C0875, Sigma-Aldrich, USA) was directly diluted to culture media at 50 µg/mL concentration. Samples were imaged at 519 nm excitation and 535 nm emission.
- Alizarin Red (A5533, Sigma-Aldrich, USA) was first diluted in NaOH and added to culture media at 100 µg/mL concentration. Samples were imaged at 578 nm excitation and 641 nm emission.

For **Study III**, only Calcein was used to stain different scaffolds on days 7, 14, 21 and 28. This technique allows for easy monitoring of mineralisation without having to distinguish between different stains within the same sample. However, it greatly increases the sample size.

For **Studies IV**, Calcein was added on day 14, scaffolds were then washed and cultured with fresh media, and Alizarin Red was added on day 21. This approach allowed for monitoring of mineralisation progress within the same samples.

For **Study V**, Calcein was added on day 18 in triplicates of as-built (untreated) and surface treated scaffolds. In this case, fluorescent images were imported in Fiji image-processing software and converted to binary files using the Triangle Algorithm – resulting in almost identical threshold value for all images. The total calcein-stained area on top of the two scaffold types was quantified.

4.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism (GraphPad Software Inc., USA) or SPSS Statistic (IBM, USA). The significance level was set to $\alpha=0.05$. In figures, (*) indicates $p<0.05$, (**) indicates $p<0.01$, (***) indicates $p<0.001$, and no notation indicates that no difference was found ($p>0.05$). Data are presented as mean $\pm$ standard deviation.

4.8.1. Assessing the strut thickness uniformity

A major aim of the manufacturing process was to produce structures with uniform strut thickness regardless of strut inclination angle. For each nominal strut thickness, 5 sets of laser parameters were used for 5 ranges of strut inclination angles. A series of Student's t-tests were conducted to ensure that measured strut thicknesses were similar to the aimed nominal values. Tests were performed for each

range of build angles ($n=5$ struts/angle/thickness) as well as the entire sample population per nominal strut thickness ($N=20$ struts/thickness).

4.8.2. Assessing the effect of design parameters on stochastic lattice properties

A series of statistical tests were performed to demonstrate how the combination of the three design parameters (Z, d, t) affected the mechanical properties ($\rho/\rho_s, E, \sigma_u$) of stochastic lattice structures.

- Three-way analysis of variance (3-way ANOVA) was performed to prove that each design parameter as well as their combination independently affects each one of the three examined mechanical properties. Hence, the 3-way ANOVA was conducted three times, one for each mechanical property as the dependent variable.
- Pearson's correlation was used to demonstrate the magnitude and direction of the potential effect of each design parameter on each mechanical property.
- Regression models were computed to correlate the design parameters with the resultant mechanical properties.

This analysis was carried out for **Study I** with a sample size of $n=5$ specimens/design.

4.8.3. Assessing the effect of specimen dimensions on the mechanical properties

First, 1-way ANOVA was used to compare the measured strut thickness and mean surface roughness at different build angles between the four lattice topologies used in **Study II**. One random specimen was imaged per lattice topology, and a total of 20 struts were used for the analysis. This was done to verify that strut morphology was independent of lattice topology.

Then, a series of analyses were performed to assess the effect of aspect ratio, unit cell size and unit cell number on the mechanical properties (E, σ_u, σ_y). A sample size of $n=5$ specimens/design was used in each analysis.

Large-sized specimens

The effect of aspect ratio on the mechanical properties of each topology was investigated through 1-way ANOVA. Tukey's post hoc test was then used to group aspect ratios that did not differ significantly.

2-way ANOVA was used to compare the mechanical properties among the BCC lattices built with different aspect ratios and unit cell sizes (i.e., unit cell edge length), followed by Tukey's post hoc test to see the effect of aspect ratio and unit cell size.

Small-sized specimens

2-way ANOVA was used to assess the effect of aspect ratio and the number of cells across the smallest edge of coupons. Tukey's post hoc test was used for pairwise comparisons.

4.8.4. Assessing the effect of design parameters on osteogenesis

Differences between measured values were assessed using either a 1-way, 2-way or 3-way ANOVA followed by Tukey's *post hoc* test. For **Study III**, connectivity, strut density and day of culture were the independent variables depending on the assay. For **Study IV**, the independent variables were the two strut thicknesses that were used (170 μm and 300 μm) and the day of culture. Sample sizes of $n=3$ or $n=4$ (specimens per design per testing day) were used depending on the assay.

4.8.5. Assessing the effect of post-processing on osteogenesis

Differences between measured values were assessed using a 2-way ANOVA followed by Tukey's *post hoc* test. The independent variables were the treatment condition (treated or untreated) and the day of culture. Last, an independent t-test was used to compare the calcein-stained area on treated and untreated scaffolds. Sample size was $n=3$ in all assays.

4.9. Outline of studies

Table 4.5. Outline of tested parameters and analyses for each study			
Study No.	Type of study	Tested parameters	Analyses
I	Mechanical	▪ Connectivity ▪ Strut density ▪ Strut thickness	▪ μCT for morphological evaluation ▪ SEM for morphological evaluation ▪ Quasi-static compression ▪ Cyclic compression
II	Mechanical	▪ Aspect ratio ▪ Topology ▪ Size	▪ μCT for morphological evaluation ▪ SEM for morphological evaluation ▪ Quasi-static compression
III	Biological	▪ Connectivity ▪ Strut density	▪ μCT for morphological evaluation ▪ SEM for morphological evaluation ▪ Cell adhesion (CCK8) ▪ Cell viability (fluorometric assay) ▪ Cell viability (live/dead staining) ▪ ECM formation (Picrosirius Red staining) ▪ Mineralisation (Calcein staining) ▪ ALP in supernatants (fluorometric assay) ▪ Expression of osteogenic genes (RT-qPCR)
IV	Biological	▪ Strut thickness	▪ μCT for morphological evaluation ▪ SEM for morphological evaluation ▪ Cell viability (fluorometric assay) ▪ Cell viability (live/dead staining) ▪ Mineralisation (calcein & alizarin staining) ▪ ALP in cell lysates (fluorometric assay) ▪ Expression of osteogenic gene (ELISA)
V	Mechanical Biological	▪ As-built ▪ Surface treated	▪ μCT for morphological evaluation ▪ SEM for morphological evaluation ▪ Quasi-static compression ▪ Cyclic compression ▪ Cell viability (fluorometric assay) ▪ Mineralisation (calcein staining) ▪ ALP in supernatants (fluorometric assay)

5. The effect of design parameters of stochastic lattices on mechanical behaviour

5.1. Structural and morphological evaluation of manufactured specimens

The thickness of 230 μm and 300 μm nominal struts were $227.8 \pm 10.5 \mu\text{m}$ and $305.4 \pm 16.9 \mu\text{m}$, respectively. Measured thicknesses were the same as the nominal values (p-values > 0.05) for all inclination angles (data provided in **Table A.2** in **Appendix**).

The relationship between relative density ρ/ρ_s and strut thickness/length ratio t/L is shown in **Figure 5.1**. The data show a clear relationship between ρ/ρ_s and t/L as expected by the Gibson-Ashby theory (105), however, the relationship is distinctly different for different connectivity values. This outcome suggests that connectivity regulates relative density independently from the other two design parameters, and generally that topology and relative density are inextricable (105).

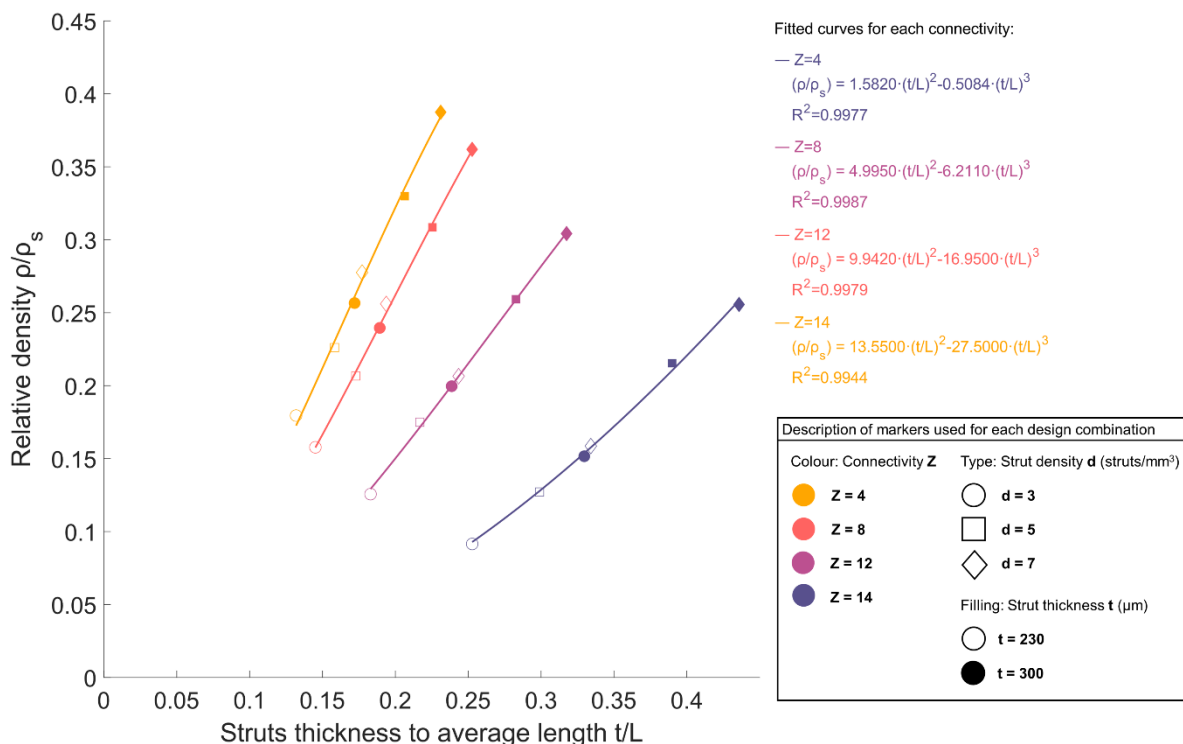


Figure 5.1. Effect of strut thickness-to-length ratio on the relative density of stochastic structures.
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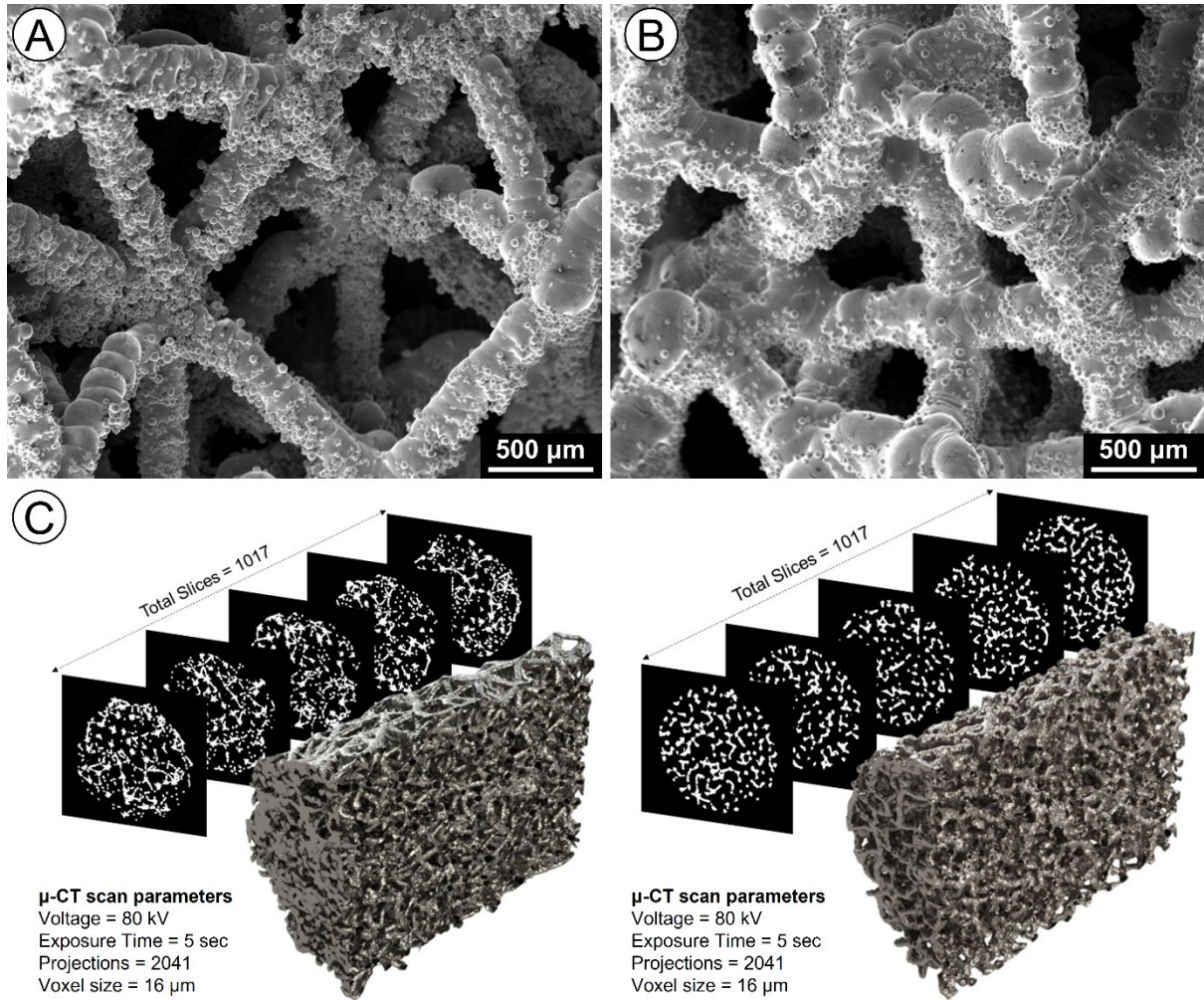


Figure 5.2. SEM and μ CT images for the morphological evaluation of stochastic structures. *Imaged structures had (A) $Z=12$ and $t=230\ \mu\text{m}$ and (B) $Z=4$ and $t=300\ \mu\text{m}$. (C) 3D representations of the scanned specimens demonstrate the internal geometry. Both specimens had strut density $d=5$. Figure reproduced under CC BY license from (109).*

Representative SEM images of lattices made with 230 μm and 300 μm struts shown in **Figure 5.2** allowed for a first (qualitative) examination of the additively manufactured surfaces. Firstly, struts of all angles appeared to have successfully been built for both nominal thicknesses. Secondly, defects commonly found on unsupported overhanging surfaces built via powder bed fusion were apparent in both cases. These defects consist of semi-sintered powder particles around the struts and nodes, dross formation at the downward-facing surfaces owing to excessive melted material (201), and texture lines and waviness at the upward-facing surfaces due to the overlap of consecutive melt-pools. Thirdly, a noticeable difference between low and high-connectivity structures was observed, with nodes in the high-connectivity lattices appearing bulkier and having more attached semi-sintered particles.

All of these morphological irregularities contribute to increased surface roughness, which affects dimensional accuracy and reproducibility. Moreover, they can serve as potential crack initiation and propagation sites under cyclic loading, thereby compromising the fatigue life of the structure. Additionally, in the context of implantable devices, semi-sintered particles on the surface of lattice structures pose a risk of detachment during implant insertion or under cyclic loading. This detachment

could trigger an inflammatory response in the surrounding tissue, an outcome that has been linked to implant loosening in both dental and orthopedic applications (337).

5.2. Quasi-static performance

Representative stress-strain curves from the uniaxial compression of structures with different connectivity ($Z = 4, 8, 12, 14$), but fixed strut density ($d=5$) and strut thickness ($t=230\text{ }\mu\text{m}$) are shown in **Figure 5.3a**. The same data for a fixed strut thickness of $t=300\text{ }\mu\text{m}$ is shown in **Figure 5.3b**. From the graphs, it can be inferred that connectivity Z altered the mechanical behaviour of the lattice structures. Clear stretch and bend-dominated characteristics are observed for lattices designed with high ($Z \geq 8$) and low ($Z=4$) connectivity levels respectively. For high connectivity, the ultimate compressive strength and stiffness are distinguishably greater than low connectivity structures. Also, all high connectivity structures exhibited post-yielding softening which was followed by a fluctuating plateau region (strain range between 20% and 30%). On the contrary, low connectivity structures exhibited an almost constant plateau stress without any post-yield softening. These findings are in line with the theoretical graphs of stretching and bending-dominated behaviours found in literature (338,339). Although the magnitude of stress was different between the $230\text{ }\mu\text{m}$ and $300\text{ }\mu\text{m}$ struts, the shape of the stress strain curves was similar for the same connectivity. These data indicate that different Z values alter the topology of stochastic lattices similarly to what has been demonstrated for periodic lattices (111,113,340).

Although tests were carried out until 40% strain, all high connectivity structures ($Z \geq 8$) failed at 30% strain with a diagonal shear band (**Figure 5.3**). On the other hand, structures with $Z=4$ failed under extended densification (macroscopically displayed as barrelling) without an apparent shear band that bisected the structures. Failure under diagonal shear bands has been reported for numerous (stretch-dominated) periodic lattices, while densification and row-by-row collapse have been linked mainly to foams or low connectivity periodic lattices owing to struts extended bending (339,341,342).

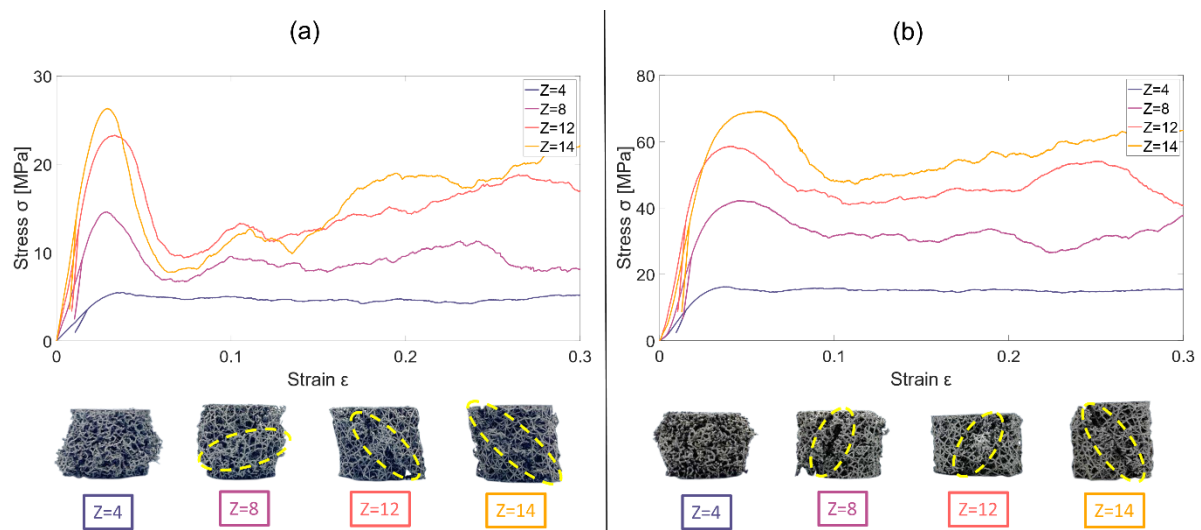


Figure 5.3. Effect of Z and t on the stress-strain curves of stochastic structures. Representative curves of structures with t equal to (a) $230\text{ }\mu\text{m}$ and (b) $300\text{ }\mu\text{m}$. Strut density was $d=5$ for all. Embedded images demonstrate each specimen type at the end of the test. Yellow ellipses indicate macroscopic crush bands in specimens with $Z \geq 8$, specimens with $Z=4$ found to fail through extended plastic densification. Figure reproduced under CC BY license from (109).

Measured mechanical properties from all quasi-static tests are summarised in **Table 5.1**. When examining the effect of design parameters on mechanical properties (relative density, elastic modulus, strength), the p-value was below 0.05 for all the independent variables (connectivity, strut density, strut thickness), when examined separately and in pairs, indicating they independently influence the mechanical properties. Results are provided in **Tables A.4-A.6** in the **Appendix**.

Table 5.1. Measured properties of stochastic structures for combinations of Z, d, and t.						
<i>Properties are referred to as: relative density ρ/ρ_s, elastic modulus E, ultimate compressive strength σ_u. Values are presented as mean $\pm$ standard deviation of the mean ($n=5$ per combination).</i>						
Design parameters	$t = 230 \mu\text{m}$			$t = 300 \mu\text{m}$		
	ρ/ρ_s [%]	E [MPa]	σ_u [MPa]	ρ/ρ_s [%]	E [MPa]	σ_u [MPa]
Z=4 – d=3	9.1 $\pm$ 0.3	140.3 $\pm$ 11.0	2.2 $\pm$ 0.1	15.5 $\pm$ 0.1	529.5 $\pm$ 43.0	7.3 $\pm$ 0.3
Z=4 – d=5	12.7 $\pm$ 0.1	375.8 $\pm$ 29.1	5.5 $\pm$ 0.2	21.5 $\pm$ 0.4	1319.3 $\pm$ 77.8	16.9 $\pm$ 1.0
Z=4 – d=7	15.9 $\pm$ 0.5	605.0 $\pm$ 21.1	8.4 $\pm$ 0.2	25.6 $\pm$ 0.4	2156.8 $\pm$ 116.1	26.3 $\pm$ 0.4
Z=8 – d=3	12.6 $\pm$ 0.2	711.1 $\pm$ 42.5	8.1 $\pm$ 0.3	20.0 $\pm$ 0.4	1791.3 $\pm$ 175.2	22.0 $\pm$ 0.6
Z=8 – d=5	17.5 $\pm$ 0.3	1449.8 $\pm$ 99.2	14.5 $\pm$ 0.6	25.9 $\pm$ 0.4	3823.7 $\pm$ 138.8	41.6 $\pm$ 0.8
Z=8 – d=7	20.7 $\pm$ 0.9	2149.2 $\pm$ 149.9	22.1 $\pm$ 0.6	30.4 $\pm$ 0.7	5146.8 $\pm$ 179.4	61.5 $\pm$ 0.7
Z=12 – d=3	15.8 $\pm$ 0.4	1146.2 $\pm$ 90.9	12.6 $\pm$ 0.6	24.0 $\pm$ 0.5	2850.1 $\pm$ 153.9	32.4 $\pm$ 0.8
Z=12 – d=5	20.7 $\pm$ 0.7	2268.2 $\pm$ 121.0	22.4 $\pm$ 0.6	30.9 $\pm$ 0.3	5835.1 $\pm$ 269.5	58.5 $\pm$ 0.4
Z=12 – d=7	25.6 $\pm$ 0.6	3182.5 $\pm$ 170.3	31.3 $\pm$ 2.0	36.2 $\pm$ 0.4	7852.5 $\pm$ 370.4	88.7 $\pm$ 1.8
Z=14 – d=3	17.9 $\pm$ 0.4	1435.5 $\pm$ 126.4	14.5 $\pm$ 0.7	25.7 $\pm$ 0.4	3619.5 $\pm$ 279.6	40.3 $\pm$ 1.6
Z=14 – d=5	22.6 $\pm$ 0.4	2509.7 $\pm$ 128.1	26.2 $\pm$ 0.3	33.0 $\pm$ 0.3	6157.3 $\pm$ 361.3	67.9 $\pm$ 1.8
Z=14 – d=7	27.8 $\pm$ 0.6	3725.7 $\pm$ 108.2	38.5 $\pm$ 0.4	38.7 $\pm$ 0.4	8178.6 $\pm$ 484.4	97.9 $\pm$ 1.3

Pearson's correlation results demonstrated that all mechanical properties (relative density, elastic modulus, strength) increase with increasing connectivity, strut density and strut thickness ($r > 0.5$) (**Table 5.2**). Results also demonstrated that the three mechanical properties tend to have a proportional relationship when examined pairwise ($r > 0.5$), however, this interrelationship is already known from the Gibson-Ashby model.

Table 5.2. Pearson's correlations for the design parameters and mechanical properties			
	Independent variables (design parameters)		
	Connectivity Z	Struts density d	Struts thickness t
Relative density ρ/ρ_s	0.563*	0.550*	0.603*
Elastic modulus E	0.589*	0.470*	0.547*
Ultimate strength σ_u	0.544*	0.472*	0.581*

(*) indicates that correlation is significant at the 0.01 level (1-tailed tests with $N=120$).

The correlations found in **Table 5.2** indicate that a multivariable regression model can be used to correlate the three design parameters with the measured relative densities. The Gibson-Ashby model would suggest that higher-order terms might better represent the physical relationship between the design parameters and relative density. However, the different relationships between connectivity levels Z and cellular dimensions shown in **Figure 5.1** made it difficult for a unified relationship to be distinguished. A generic multivariable linear regression model was found adequate (adjusted $R^2=98.15\%$) to predict relative density using the three design parameters of this study:

$$\rho/\rho_s = 0.0110 \cdot Z + 0.0252 \cdot d + 1.29 \cdot t - 0.344$$

Where, Z corresponds to nodal connectivity [struts/node], d to strut density [struts/mm³], and t to strut thickness [mm]. This relationship allows the relative density of a stochastic structure (and subsequently its stiffness and strength) to be predicted when the structure is being designed.

Power-law regression models were used to link modulus to relative density according to Gibson-Ashby (**Figure 5.5**). Two power-law fits were required to correlate elastic modulus to relative density for high ($Z \geq 8$) and low connectivity structures ($Z=4$). The power indices n_2 calculated for both high and low connectivity structures were greater than 2, indicating all structures had theoretically bending-dominated characteristics according to the Gibson-Ashby model, contrariwise to what was observed by the stress-strain curves or by the Maxwell criterion (**Table 4.1**).

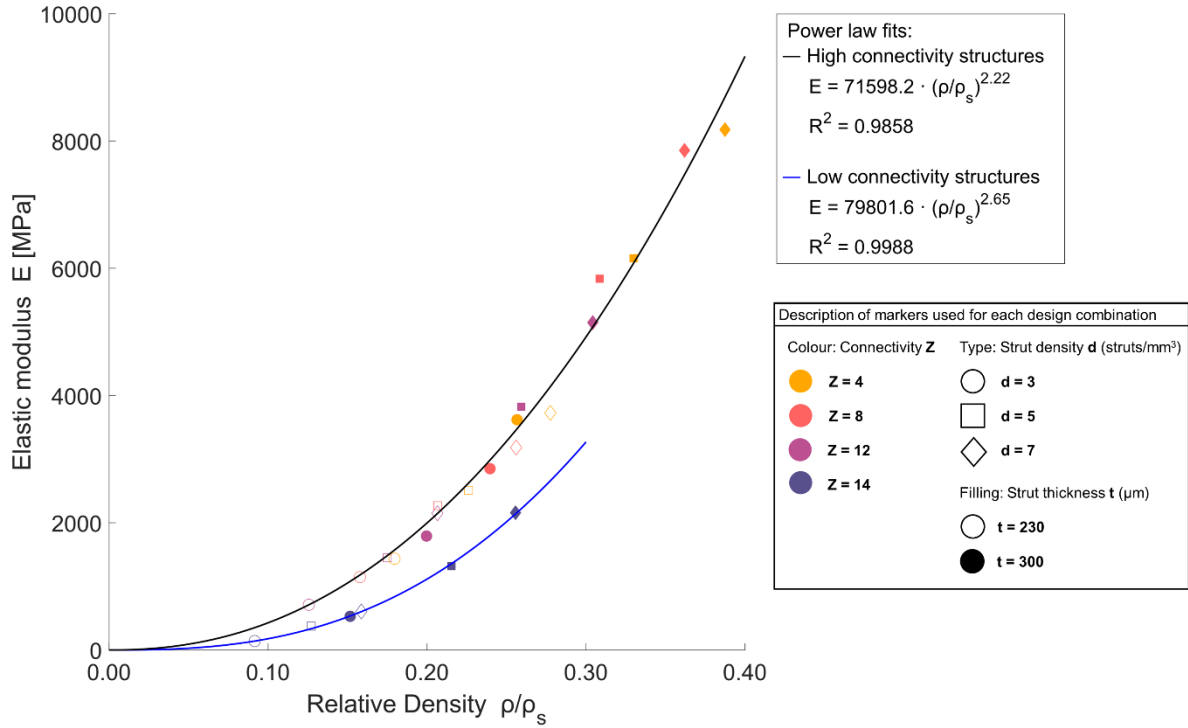


Figure 5.4. Effect of relative density on the modulus of stochastic structures. Each point in the graph corresponds to the average of 5 measurements. Figure reproduced under CC BY license from (109).

5.3. Fatigue performance

All structures exhibited failure mechanisms which have been previously reported in compression-compression fatigue testing of both metallic foams and lattices. The initial increase in strain at the start of the test was followed by an incubation period, where cracks are usually starting to be formed, while plastic strain remained constant over increasing cycles (plateau region) (**Figure 5.5**). The plateau region ended up to a knee point, after which, plastic strain started to accumulate rapidly until catastrophic failure. This steep increase in strain can be observed as either a vertical line with few “jumps” or as a straight vertical line (172,342). The former, which was exhibited in almost all structures with $Z=4$ and in some with $Z=8$, indicates that distinct crush bands were formed in different weak regions inside specimens’ volume. While the latter, found in all high connectivity structures, indicates a uniform deformation seen as a single macroscopic crush band that extended across the diagonal of the lattice specimens.

The developed crush bands sheared the middle part of struts or ran through strut junctions at nodes. SEM images reveal that struts experienced a ductile failure with the generation of numerous dimples at the fracture surface (**Figure 5.6A,C**), indicative of extended plastic deformation during cyclic loading. On the contrary, images at the vicinity of nodes reveal characteristics of intergranular fracture indicative of brittle failure (**Figure 5.6B,D**). This can be attributed to the stress concentration at the junction of nodes leading to rapid crack propagation.

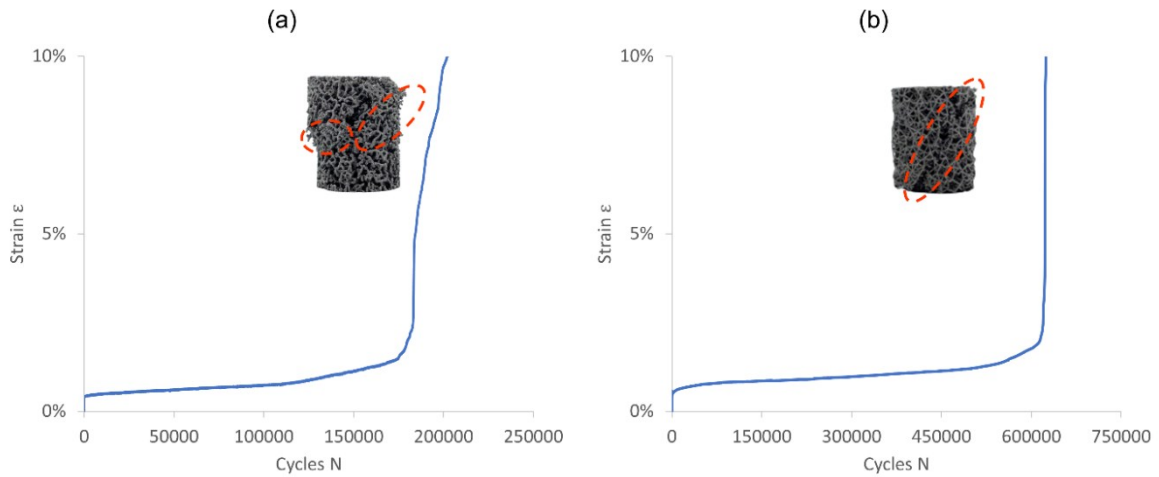


Figure 5.5. Strain accumulation over compression cycles in stochastic structures. Representative plots for structures with (a) $Z=4$ and $t=300\ \mu\text{m}$ and (b) $Z=12$ and $t=230\ \mu\text{m}$. Embedded images demonstrate each specimen at the end of the test. Red ellipses indicate crush bands which led to catastrophic failure. Test level 25% of the ultimate compressive strength in both structures. Figure reproduced under CC BY license from (109).

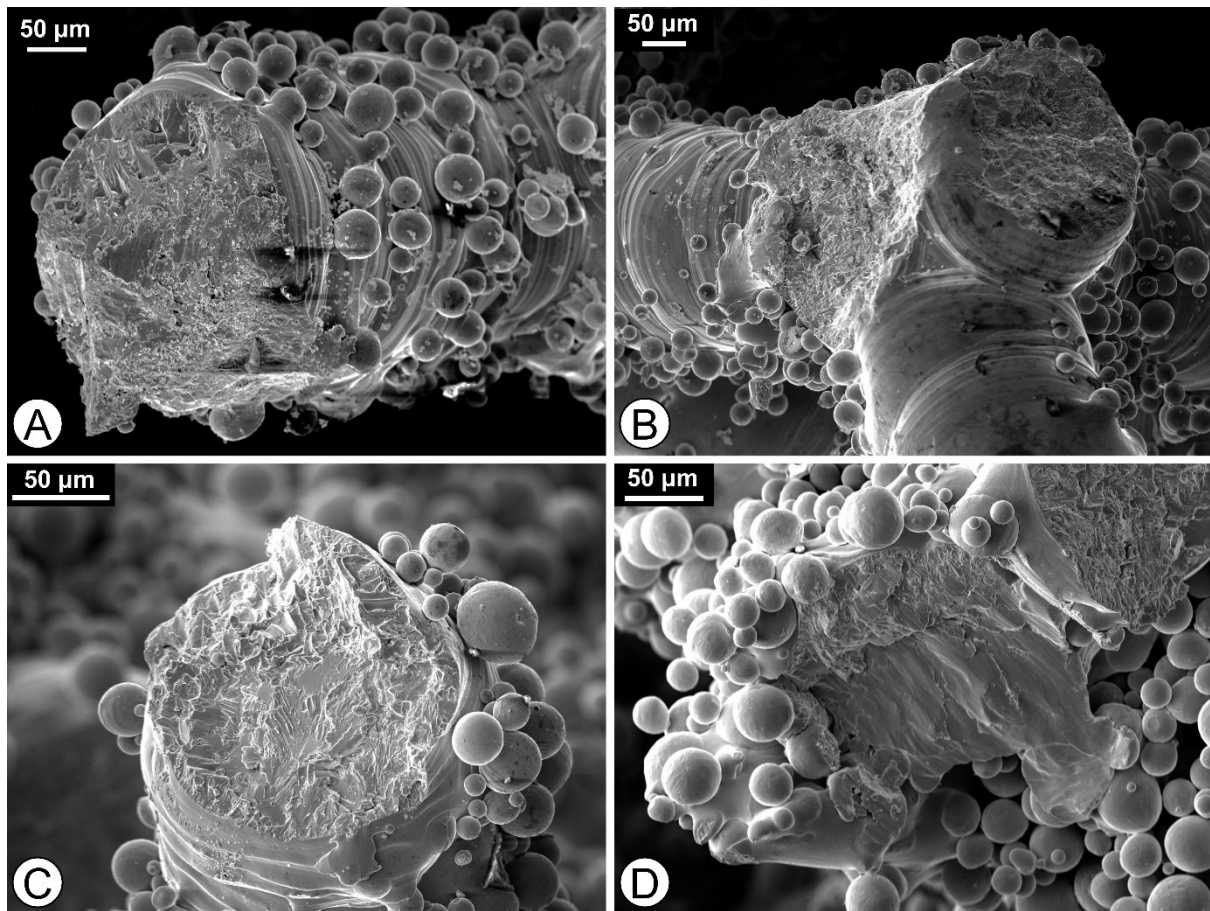


Figure 5.6. SEM images of fractured lattice features after fatigue testing. Fracture planes of individual struts (A,C) and strut junctions at nodes (B,D). Images A and B correspond to the lattice with $Z=4$ and $t=300\ \mu\text{m}$. Images C and D correspond to the lattice with $Z=12$ and $t=230\ \mu\text{m}$. Both imaged specimens were tested at test level 35%.

The S-N curves and fatigue strength data are shown in **Figure 5.7** and **Table 5.3** respectively. Comparing the four datasets with 230 μm thick struts, increasing the connectivity from $Z=4$ to $Z=14$ increased the relative density of the structures from 12.1% to 21.1%, increased the fatigue strength from 0.95 to 5.83 MPa and increased the normalised fatigue strength (σ_f/σ_u) from 0.17 to 0.22. For the two datasets with the same relative density (21.1%), the structure with $Z=14$ had 162% of the fatigue strength of the structure with $Z=4$, but the normalised fatigue strengths were similar. These data indicate that increasing connectivity can increase the fatigue strength of a structure for a fixed relative density (or porosity). On the other hand, the lattices with equal relative density exhibited equal normalised fatigue strength which indicates that the normalised fatigue strength may be independent of topology. Subsequently, it may be possible to estimate a structure's fatigue strength during the design phase through its relative density and ultimate strength.

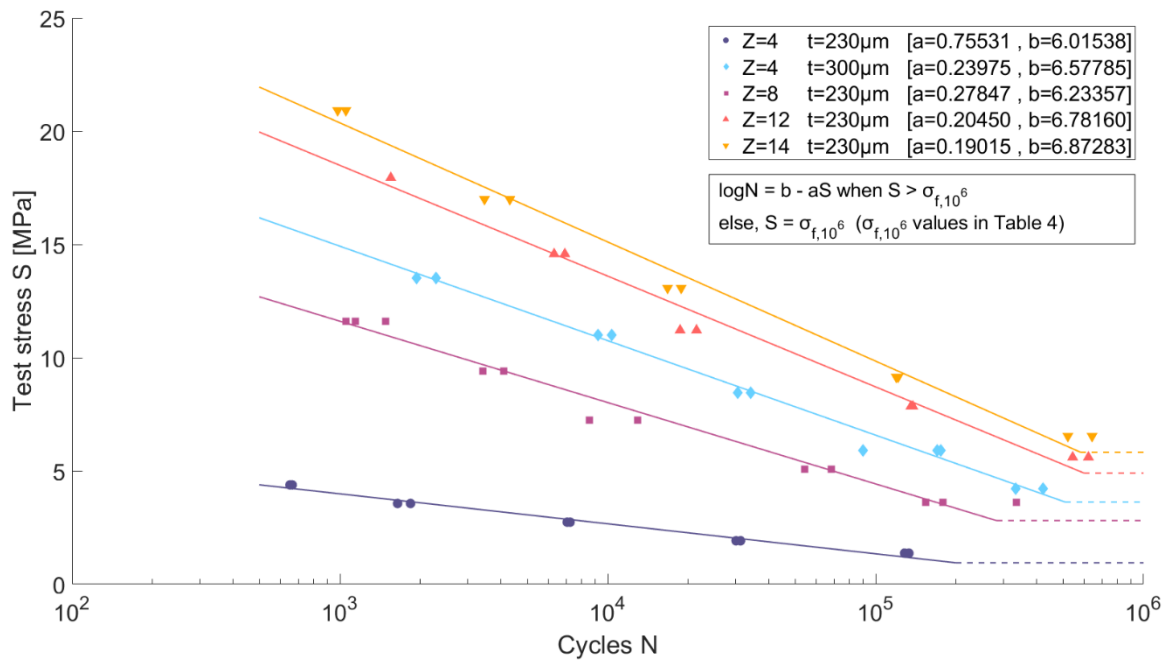


Figure 5.7. S-N curves of compression-compression fatigue tests for stochastic structures. *Strut density $d=5$ for all specimens. Dashed horizontal lines correspond to fatigue strength at 10^6 cycles, estimated using the modified staircase method ($n=6$). Figure reproduced under CC BY license from (109).*

Table 5.3. Absolute and normalised fatigue strength of stochastic structures					
	Level of nodal connectivity Z				
	$Z=4$	$Z=4$	$Z=8$	$Z=12$	$Z=14$
Nominal strut thickness [μm]	230	300	230	230	230
Relative density [%]	12.1 ± 0.3	21.1 ± 0.5	16.0 ± 0.7	19.3 ± 0.4	21.1 ± 0.8
Absolute σ_f at 10^6 cycles [MPa]	0.95 ± 0.15	3.63 ± 0.37	2.81 ± 0.16	4.91 ± 0.61	5.83 ± 0.64
Normalised σ_f at 10^6 cycles	0.17 ± 0.03	0.21 ± 0.03	0.19 ± 0.01	0.22 ± 0.03	0.22 ± 0.02

Strut density $d=5$ for all specimens. Properties are presented as mean $\pm$ standard deviation of the mean.

6. The effect of lattice dimensions on mechanical properties

6.1. Characterisation of the lattice specimens

Key structural characteristics for each lattice type are summarised in **Table 6.1**. Specimens' dimensions exhibited high consistency with less than 2% deviation from nominal values.

The first part of the study aimed to isolate the effect of aspect ratio while minimising the influence of size effects. Prior research and testing standards (133,135,136) suggest that specimens are sufficiently large for that purpose when composed of at least 7 unit cells. While this criterion is straightforward to apply to periodic structures, which were designed with a minimum of 10 unit cells along their shortest edge, it is less clear for stochastic structures, where pores are difficult to define. To address this, micro-CT analysis was used to calculate the average strut distance (i.e., trabecular separation) in the lattice specimens, which served as an approximation of the pore size of the stochastic lattices.

Strut distance and pore size are not equivalent by definition. However, even in periodic lattices, defining a single representative pore size can be challenging. For example, in the BCC topology, a pore can be approximated as the largest sphere that fits within the octahedral void formed by two consecutive unit cells. However, the case of the octet-truss topology is more complex. The octet-truss constitutes a superposition of octahedral and tetrahedral substructures, leading to a hierarchical pore distribution. As illustrated in **Figure A.3** in the **Appendix**, each unit cell comprises a large central pore from the octahedral structure and eight smaller pores from the surrounding tetrahedral substructures. Therefore, reporting a single pore size for the octet-truss lattice would be misleading.

For both lattice types, the measured strut distance was found to be within the theoretical pore size range. In the BCC lattice, where pore size is easier to interpret, the strut distance was consistently ~100 μm smaller than the theoretical pore size across all tested unit cell sizes. This underestimation arises because (a) strut distance accounts for void space near the edges of the specimen, which may not correspond to well-defined pores, and (b) surface roughness causes local deviations in strut thickness.

Nevertheless, based on these findings, strut distance can serve as a practical estimate of how many pores fit within a lattice. Since the measured strut distance was at least 10 times smaller than the shortest edge of the specimens (13 mm), the specimens were considered large enough to avoid size-related effects in mechanical testing.

All topologies were fabricated with minimal structural defects with representative images shown in **Figure 6.1**. Typical forms of surface roughness associated with powder bed fusion, such as semi-sintered particles and strut waviness, were seen in all specimens. Still, strut thickness and surface roughness (measured via μCT scans) were found to be consistent among different topologies and different build angles as aimed ($p < 0.05$; no significant different values, see **Figure A.2** in **Appendix**). Lattices of different topologies were fabricated at different batches, and this further informs that strut morphology did not exhibit batch-to-batch variation. Enclosed porosity was also found to be $< 0.01\%$ in all specimens according to micro-CT analysis. This suggests that the manufacturing process exhibited high consistency and repeatability for the fabrication of all lattice topologies.

Table 6.1. Structural characteristics of lattice types tested for different aspect ratios						
	BCC	BCC	BCC	Stochastic	Stochastic	Octet-truss
Connectivity [struts/node]	8	8	8	4	8	12
Unit cell edge l [mm]	1.0	1.5	2.0	–	–	1.3
Strut density [struts/mm ³]	8.0	2.4	1.0	3.0	3.0	11.0
Slenderness ratio t/L	0.27	0.17	0.13	0.23	0.18	0.25
Micro-deformation mode	Bending	Bending	Bending	Bending	Stretching	Stretching
Relative density [%]	20.1 ± 0.4	9.8 ± 0.3	5.8 ± 0.2	9.0 ± 0.2	11.5 ± 0.3	30.6 ± 0.3
Strut distance [mm]	0.51 ± 0.10	0.86 ± 0.16	1.30 ± 0.19	1.04 ± 0.31	1.09 ± 0.33	0.45 ± 0.18
Theoretical pore size [mm]	0.56	0.99	1.40	–	–	0.15 - 0.69 *

* In the octet-truss, the large pore size corresponds to the inscribed sphere within the octahedral void at the core of the unit cell, while the small pore size corresponds to the inscribed spheres within the tetrahedral voids at the edges of the unit cell.

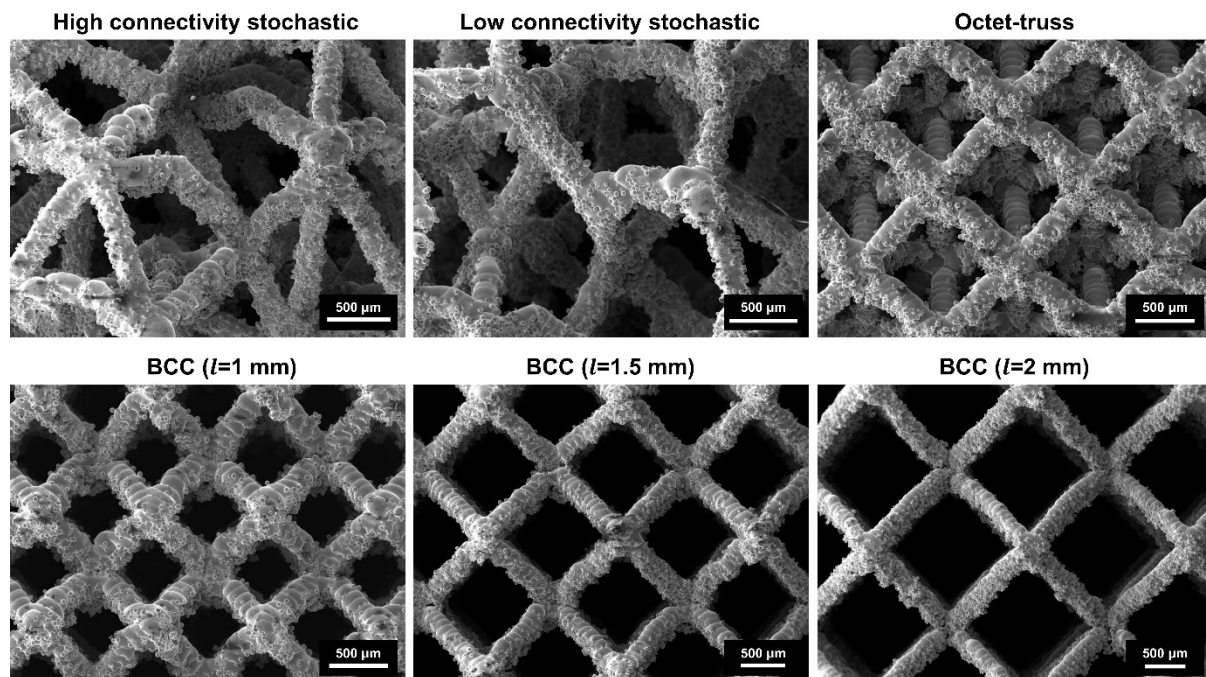


Figure 6.1. SEM images of lattice topologies used in the aspect ratio study. Figure reproduced under CC BY license from (322).

6.2. The effect of aspect ratio and strut micro-deformation mode

6.2.1. Convergence of mechanical properties in stretch and bend-dominated lattices

Measured stiffness and strength values are summarised in **Figures 6.2** and **6.3**, respectively. The aspect ratio had strong effect ($p < 0.001$) on each mechanical property (stiffness, yield, ultimate compressive strength, and energy absorption). The stochastic (stretch-dominated) lattices (see **Figure 6.2a**) demonstrated constant stiffness for $\zeta \leq 2$ ($p > 0.05$) and increased stiffness for larger aspect ratios ($p < 0.05$). On the contrary, the BCC (bend-dominated) lattices (see **Figure 6.2b**) demonstrated constant stiffness for $\zeta > 1$ ($p > 0.05$) and increased stiffness for smaller aspect ratios ($p < 0.05$).

The stochastic and BCC lattices exhibited similar trends regarding yield (**Figure 6.3a**) and compressive strength (**Figure 6.3b**). The yield and compressive strength of the stochastic (stretch-dominated) lattice reduced as ζ increased from 0.5 to 0.65 ($p < 0.01$ for yield, $p < 0.001$ for strength), but thereafter remained constant as ζ increased to 3. The yield and compressive strength of the BCC (bend-dominated) lattice reduced as ζ increased from 0.5 to 1 ($p < 0.01$ for yield, $p < 0.001$ for strength), but thereafter also remained constant as ζ increased to 3.

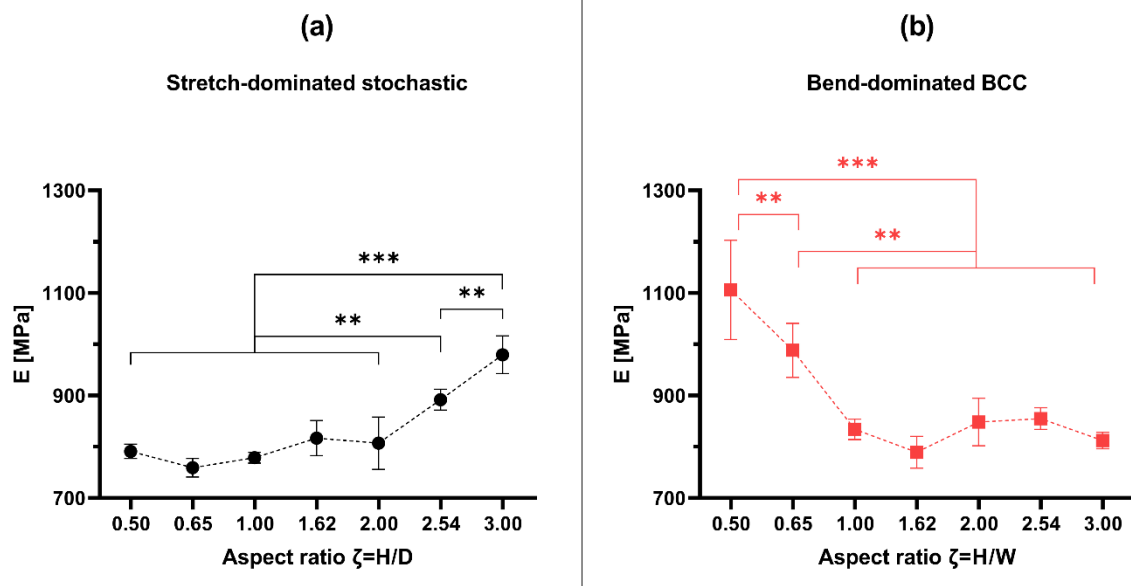


Figure 6.2. The elastic modulus of stochastic and BCC lattices for different aspect ratios. Statistical results are provided in Tables A.7 and A.8. Figure reproduced under CC BY license from (322).

In both lattice topologies, the increase in ultimate strength was more pronounced (i.e., more statistically significant) than the increase in yield. This can be attributed to the higher variation in yield measurements for specimens with same aspect ratio. Yield strength is determined using a constructed 0.2% offset line, which can introduce variability due to curve noise or minor inconsistencies in removing the toe region. In contrast, the ultimate strength is defined by a clear peak (in stochastic lattices) or the formation of a plateau stress (in BCC lattices), making it less prone to measurement uncertainty and thus more statistically powered.

Additionally, in both lattice topologies, the effect of aspect ratio on strength was relatively small (13% for the stochastic and 20% for the BCC lattices) compared to its effect on stiffness (29% for the stochastic and 40% for the BCC).

For the BCC (bend-dominated) structures, the energy absorption reduced as the aspect ratio increased from 0.5 to 1 ($p < 0.01$), but thereafter remained the same ($p > 0.05$) (**Figure 6.9**). The higher energy absorbance was related to the higher plateau stress (equivalent to the measured compressive strength) in the lower aspect ratio specimens.

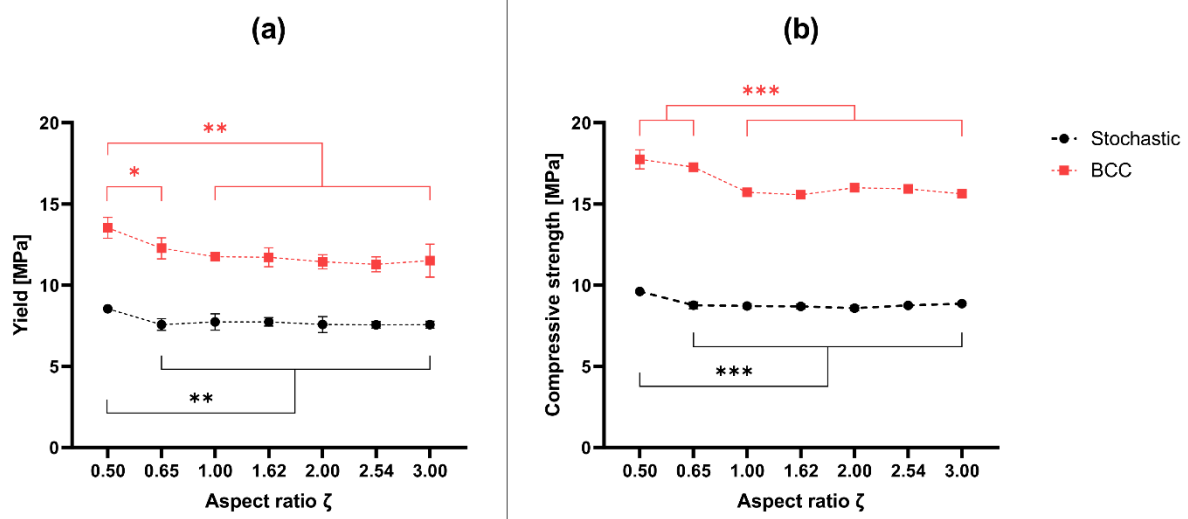


Figure 6.3. Yield and ultimate strength of stochastic and BCC lattices for different aspect ratios. No comparison between lattice types was performed. Statistical results are provided in Tables A.9 – A.12. Figure reproduced under CC BY license from (322).

Representative stress-strain curves and DIC images from the quasi-static compression tests are displayed in **Figure 6.4**. The shapes of the curves resembled well-known patterns that are associated with stretch and bend-dominated responses of lattices in the micro-scale (95). In stochastic (stretch-dominated) lattices, stress reached a peak value which was followed by stress softening owing to the localised fracture of cells through the formation of shear bands, ultimately leading to catastrophic failure. On the contrary, cells in BCC (bend-dominated) lattices tend to collapse, and structures fail in a layer-by-layer manner due to the bending of their struts, ultimately leading to structure densification.

For the stochastic (stretch-dominated) lattices with the smallest aspect ratio ($\zeta=0.5$), multiple shear bands were observed which increased the measured strength of the specimen, but not the measured stiffness. Post yield, the stress-strain curves demonstrated relatively low stress fluctuation indicating that locally collapsed regions did not merge into a catastrophic crack (as seen in **Figure 6.4**). High aspect ratio specimens (ζ equal to 2.54 and 3) progressively compressed due to the generation of multiple distinct shear bands; a major shear band was followed by secondary ones generated at distant regions along the specimens' height. This did not affect the specimen's measured strength but did increase the measured stiffness (**Figures 6.2 and 6.3**).

For the BCC (bend-dominated) lattices, high strain accumulated centrally in the low aspect ratio BCC specimens (ζ equal to 0.5 and 0.65), while the sides of the specimen experienced less strain. High strain accumulation near the platens corresponded to the collapse of cell rows in the vicinity of platens which further disrupted stress distribution towards the centre. This had the effect of increasing the measured stiffness, strength (**Figure 6.4**) and energy absorption of the specimen (**Figure 6.8**). The intermediate aspect ratio specimens ($1 \leq \zeta \leq 2$) exhibited uniform strain accumulation along the specimen's height and a macroscopic barrel shape at failure, while the high aspect ratio specimens ($\zeta > 2$) exhibited macroscopic (global) buckling behaviour with localised strains forming two symmetrical shear bands about the specimen's mid-plane. However, these phenomena for the intermediate and large aspect ratio did not affect the measured mechanical properties.

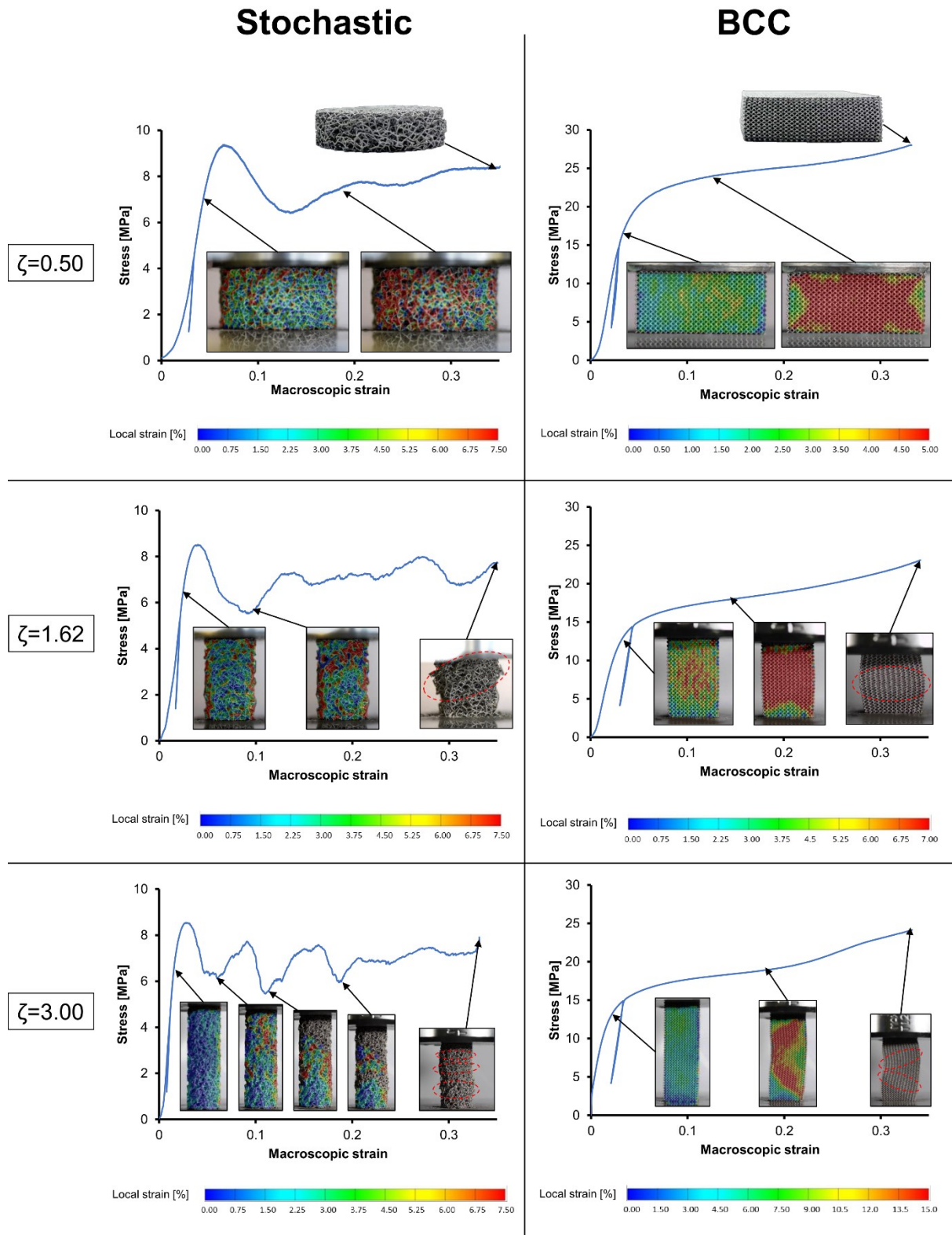


Figure 6.4. Stress-strain curves of stochastic and BCC lattices for different aspect ratios. *Embedded DIC images capture the local longitudinal stains at the point of yield and the plastic deformation region. A longitudinal strain scale is provided for each specimen. Circles indicate the fracture regions in the plain images at the end point of tests. Figure reproduced under CC BY license from (322).*

6.2.2. Further investigation for the role of micro-deformation mode

Representative stress-strain curves of the stretch dominated octet-truss lattices (**Figure 6.5a**) and the bend-dominated Z4 stochastic lattices (**Figure 6.5b**) confirm that the observations in **section 6.2.1** were not confined to particular lattice topologies. The shape of the curves as well as the failure mode of the specimens were unaffected by the change of aspect ratio in both cases.

In the octet-truss lattices, a major diagonal shear band generated a cleavage line leading to catastrophic failure in the intermediate and high aspect ratio specimens, making the structures appear more brittle (**Figure 6.5a**). In the low aspect ratio specimens, multiple shear bands at distant regions were formed which did not lead to catastrophic fracture, caused a smaller post-yield stress-drop and made the lattices appear more ductile (**Figure 6.5a**).

In the Z4 stochastic lattices, the $\zeta=0.5$ and $\zeta=1.62$ aspect ratio specimens exhibited collapsed towards the middle of the specimen (**Figure 6.5b**) as previously seen in low connectivity structures such as lattices, foams and trabecular bone cores (109,341,343). More interestingly though, the high aspect ratio ($\zeta=3$) specimens demonstrated a macroscopic buckling which originated by the collapse of two main regions along specimen's length (**Figure 6.5b**). These observations matched the ones seen previously in the bend-dominated BCC lattices.

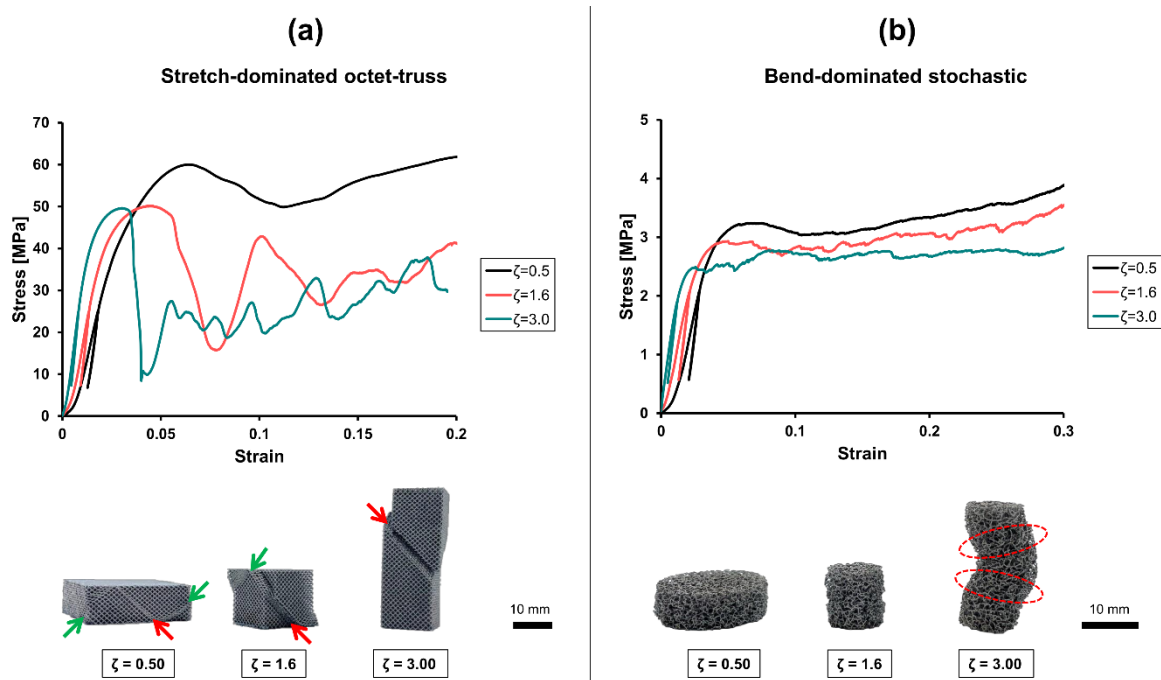


Figure 6.5. Stress-strain curves of octet-truss and Z4 stochastic lattices for different aspect ratios. Embedded images demonstrate failed specimens; red and green arrows indicate major and any secondary shear bands generated inside the octet-truss specimens. Circled regions in the stochastic lattice with $\zeta=3$ indicate local collapses that led to macroscopic buckling. Figure reproduced under CC BY license from (322).

When considering the measured mechanical properties, similarly to the stretch-dominated (Z8) stochastic lattices, the stiffness of the octet-truss lattices increased with increasing aspect ratio (**Figure 6.6a**). While, similarly to the bend-dominated BBC lattices, the stiffness of the bend-dominated (Z4) stochastic lattices increased with decreasing aspect ratio (**Figure 6.6b**). In both cases, the ultimate strength decreased with increasing aspect ratio. Although the trends in stiffness and strength were the same for the same micro-deformation mode (stretch-dominated and bend-dominated structures), the change in properties happened at different values of aspect ratio indicating the individual topologies also affected the data and appear to alter the convergence point.

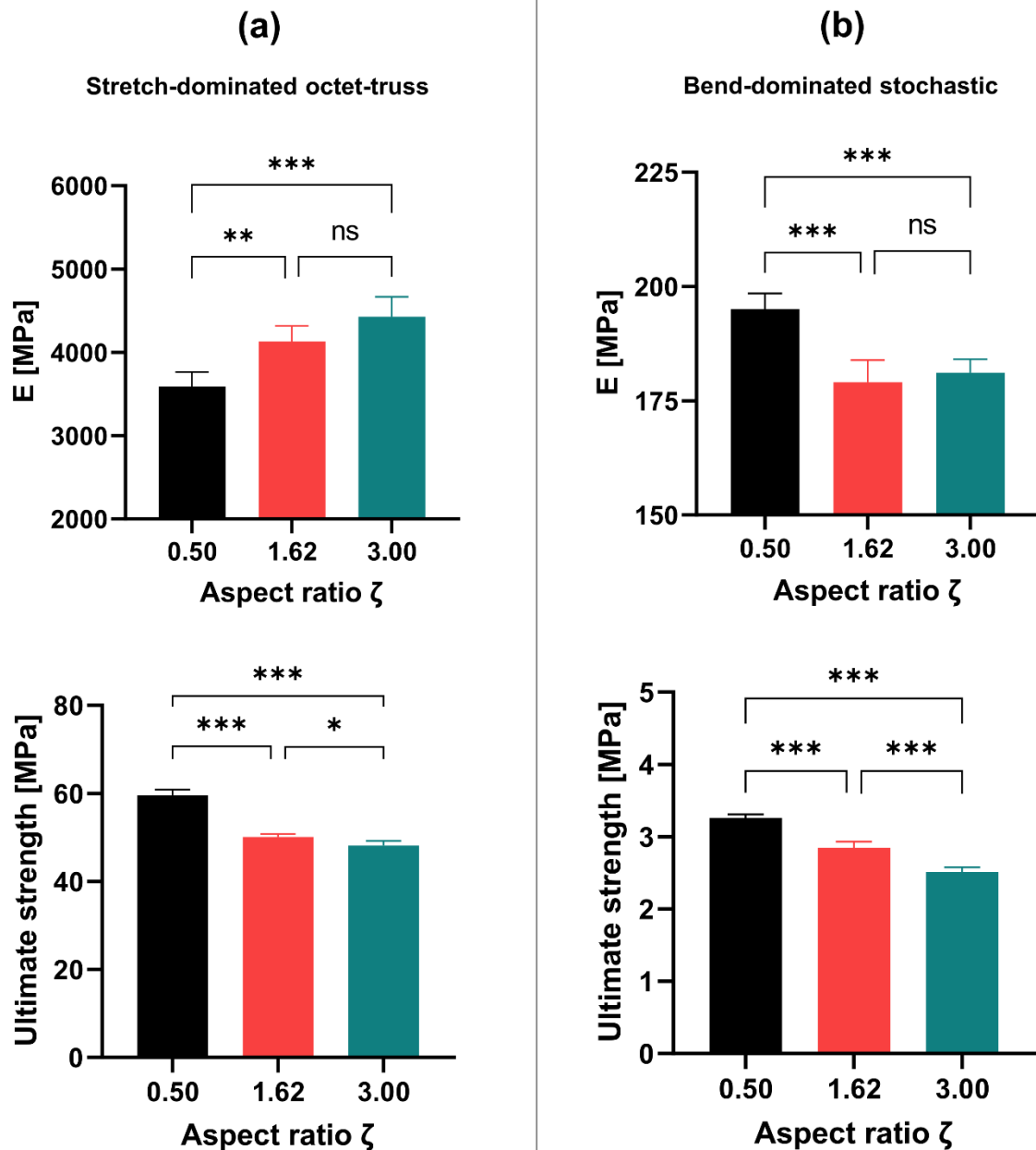


Figure 6.6. Elastic modulus and ultimate strength of octet-truss and Z4 stochastic lattices for different aspect ratios. Statistical results are provided in Tables A.13-A.16. Figure reproduced under CC BY license from (322).

6.3. The effect of aspect ratio and relative density

Two additional (highly porous) BCC lattice structures with relative densities of 6% and 10% were tested. Both structures demonstrated similar shapes of stress-strain curves with the first BCC lattice (edge length 1 mm and relative density 20%). Representative curves for three different aspect ratios (ζ equal to 0.5, 1.6 and 3.0) are shown in **Figure 6.7**. In addition, both types of the highly porous BCC lattices demonstrated similar macroscopic failure patterns with the BCC lattices tested in **section 3.2**. In particular, the high aspect ratio specimens ($\zeta=3.0$) exhibited two main regions of high densification which led to a macroscopic buckling (**Figure 6.8**). The specimens with the intermediate aspect ratio ($\zeta=1.6$) exhibited a primary densification in the middle of the structure (**Figure 6.8**). While the low aspect ratio specimens ($\zeta=0.5$) exhibited highly gradient local deformation at two sides of the specimen. One side displayed a collapse at the site of the moving platen and the other side a shear band collapse which led the middle of the specimen intact.

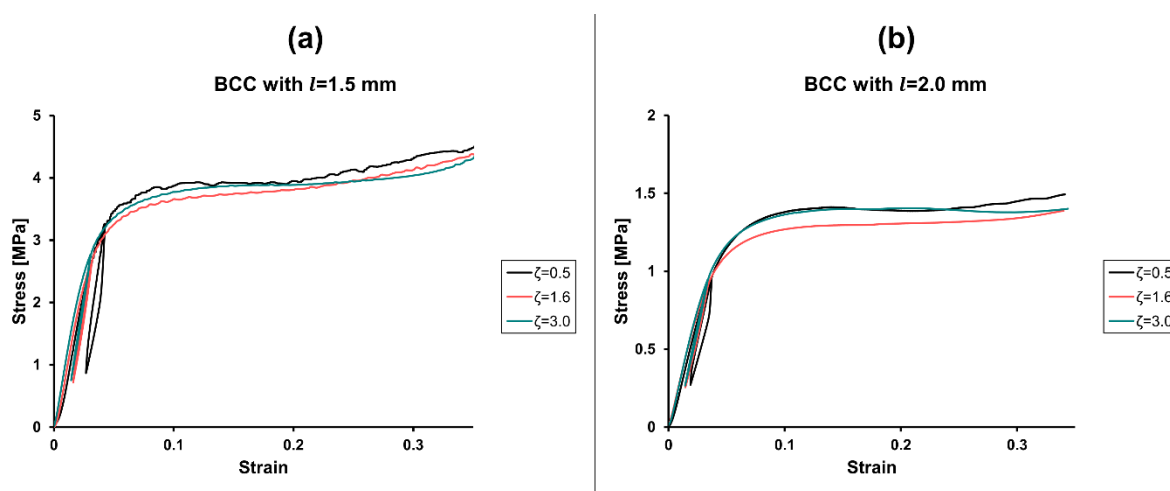


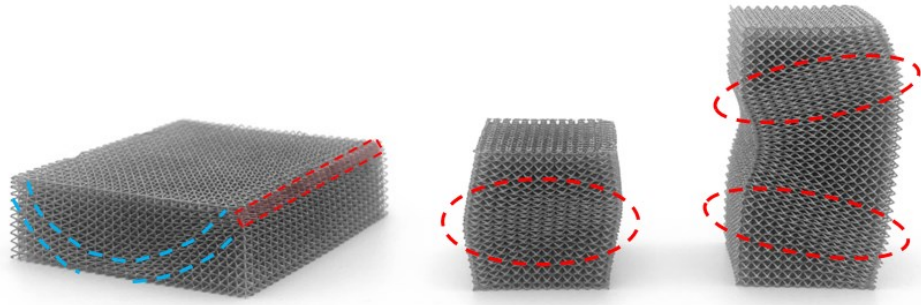
Figure 6.7. Stress-strain curves of BCC lattices with different unit cell sizes and aspect ratios. *Figure reproduced under CC BY license from (322).*

While these observations are highly in line with the ones seen at the BCC of **section 6.2.1**, the effect of aspect ratio in the mechanical properties was seen to attenuate with decreasing relative density (**Figure 6.9**). Similar to all previously tested structures, compressive strength was found to increase when $\zeta=0.5$ for both highly porous BCC lattices (11% increase when $l=1.5$ mm and 8% increase when $l=2$ mm). Furthermore, the BCC lattice with 10% relative density exhibited almost identical trends of increasing stiffness and energy absorption with decreasing aspect ratio as seen in the BCC lattice with 20% relative density. On the contrary, no statistical difference was seen in the stiffness and energy absorption of the BCC with 6% relative density for different aspect ratios.

Overall, statistical analysis revealed that aspect ratio and relative density had a coupled effect on all mechanical properties that were investigated ($p < 0.001$ for the interaction in **Tables A.17-A.19** in the **Appendix**).

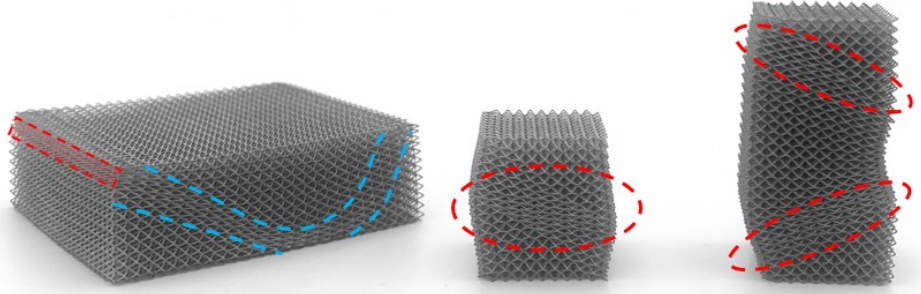
(a)

$l = 1.5 \text{ mm}$



(b)

$l = 2.0 \text{ mm}$



10 mm

$\zeta=0.5$

$\zeta=1.6$

$\zeta=3.0$

Figure 6.8. Images of BCC lattices made with different unit cell sizes following compression testing. Specimens with $\zeta=1.6$ and $\zeta=3.0$ are shown from the front, with red circles indicating the locations of generated shear bands. The specimen with $\zeta=0.5$ is shown at an angle to highlight its multi-modal failure pattern. Red-marked regions indicate layer collapse near the platen on the lateral side, while blue-marked regions highlight localised shear band collapse at the front plane. Figure reproduced under CC BY license from (322).

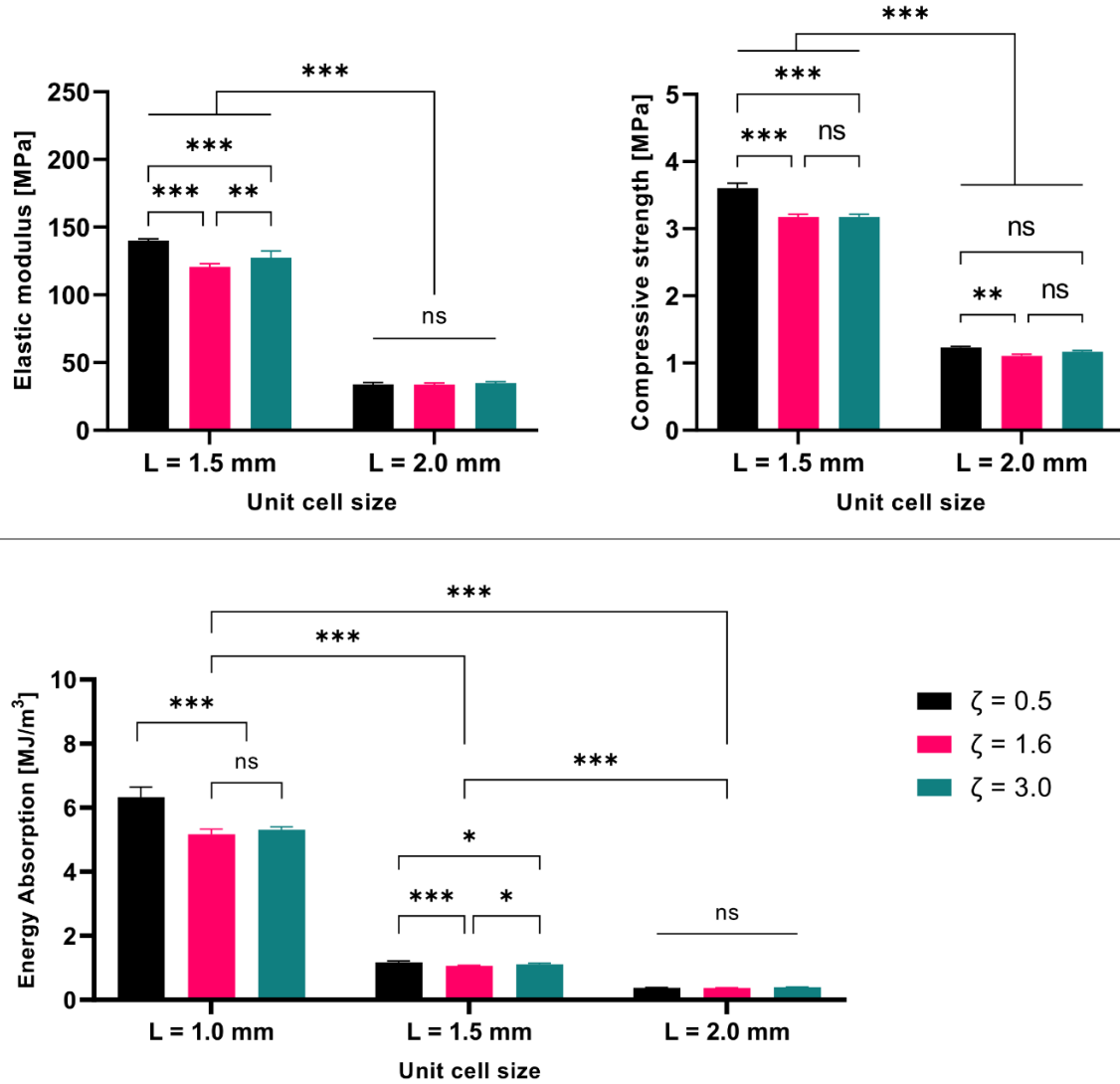


Figure 6.9. Elastic modulus, compressive proof strength and energy absorption for different BCC lattice unit cell sizes. Statistical results are provided in Tables A.17-A.19. Figure reproduced under CC BY license from (322).

6.4. The effect of aspect ratio and lattice size

Additional small-sized specimens of the bend-dominated BCC and the stretch-dominated octet-truss were tested to investigate the interplay between aspect ratio, topology, and size. The small-sized specimens were designed by stacking 3 and 5 across the smallest edge depending on the aspect ratio.

Both lattice types demonstrated similar morphologies of the stress-strain curves with the large-sized specimens, suggesting that the bend-dominated and stretch-dominated behaviours were maintained even under the influence of size effects. The small-sized octet specimens failed through the formation of shear bands (**Figure 6.10**) similar to what was observed in the large-sized specimens. Again, the low-aspect-ratio ($\zeta=0.5$) specimens demonstrated higher toughness than the specimens with $\zeta=1.6$ and $\zeta=3.0$ which most of the time failed catastrophically at low plastic strains. In analogy, the small-sized BCC lattices exhibited similar failure patterns as the large-sized specimens, with densification when $\zeta=0.5$ and $\zeta=1.6$ or macroscopic buckling when $\zeta=3$ (**Figure 6.11**).

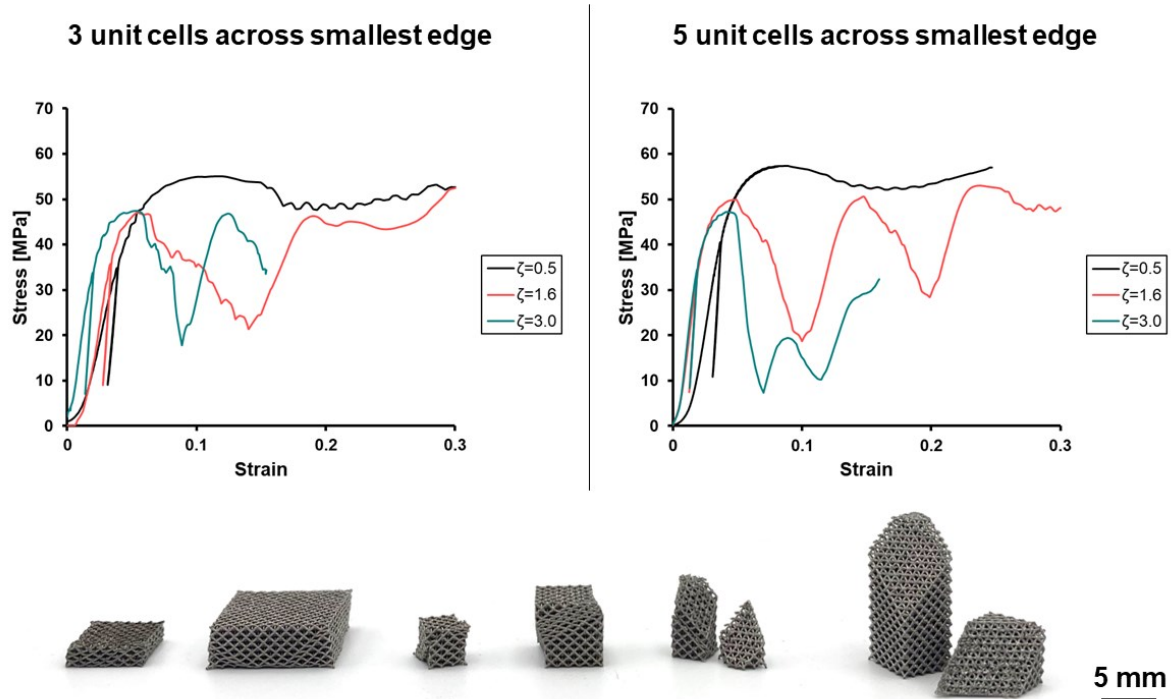


Figure 6.10. Stress-strain curves and images of fractured small-sized octet-truss lattices.

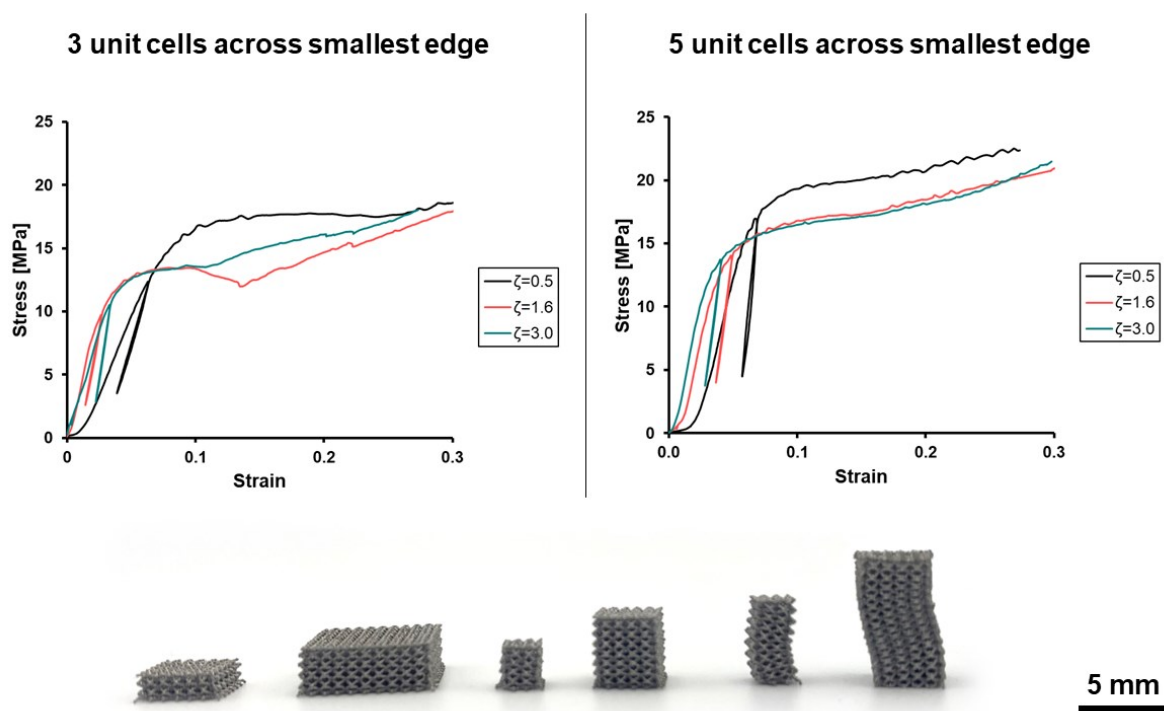


Figure 6.11. Stress-strain curves and images of fractured small-sized BCC lattices.

Aspect ratio and size appeared to have a joint effect on the elastic modulus of octet-truss and BCC lattices but with deviating effects depending on topology.

In the stretch-dominated octet-truss (**Figure 6.12a**), no statistical differences were seen between the moduli of different-sized specimens when designed with the same aspect ratio, but only for $\zeta=1.6$ and 3, indicating the absence of size effects. When the octet-truss was built with 5 unit cells across the smallest edge, a sudden increase in modulus was recorded for the low aspect ratio specimens ($\zeta=0.5$) which was higher than any specimen of the same aspect ratio ($p<0.001$) or size ($p<0.05$). For the rest of the aspect ratios, moduli values were similar to the different-sized specimens for fixed ζ .

In the bend-dominated BCC (**Figure 6.12b**), when specimens were composed of ≥ 5 unit cells, the elastic modulus appeared to increase for decreasing aspect ratio as seen in the large-sized specimens. In addition, the modulus values for the same aspect ratio were similar when specimens were composed of ≥ 5 and ≥ 10 unit cells. On the contrary, when BCC was composed of ≥ 3 unit cells, the elastic modulus decreased for decreasing aspect ratio. More interestingly, while the $\zeta=3$ specimens had similar modulus with the specimens made with ≥ 5 and ≥ 10 unit cells, the $\zeta=0.5$ and $\zeta=1.6$ had significantly lower moduli ($p<0.01$) for the respective specimens made with ≥ 5 and ≥ 10 unit cells. This indicates that in the case of BCC, size effects are influenced by aspect ratio and the number of unit cells stacked along the loading direction.

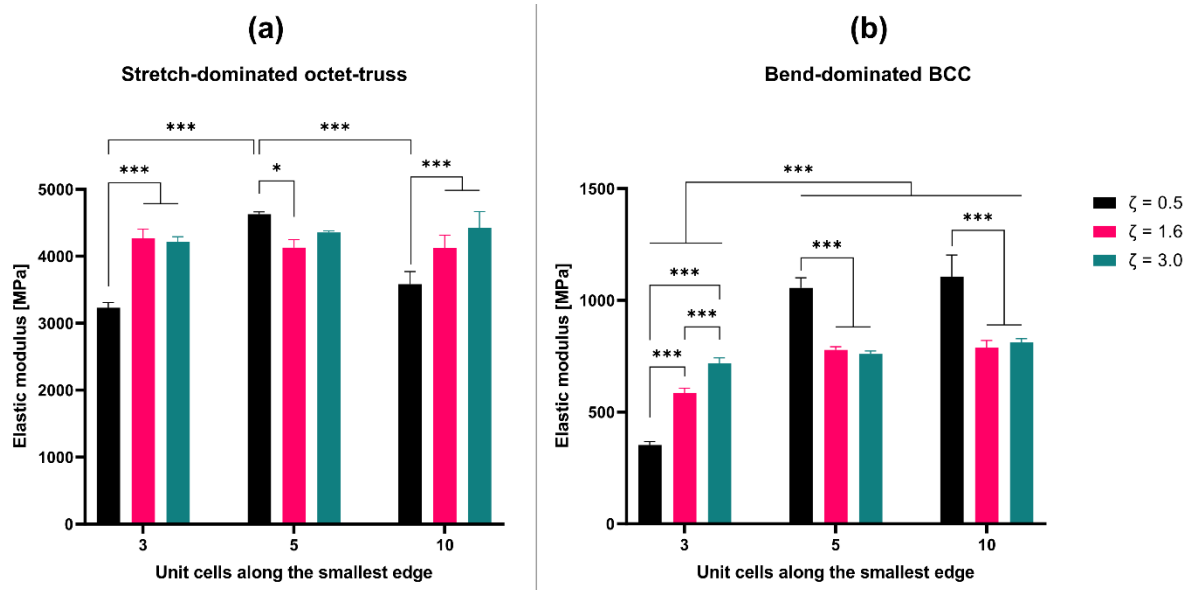


Figure 6.12. Effect of aspect ratio and specimen size on the elastic modulus of octet-truss and BCC lattices. Statistical results are provided in Tables A.20 and A.21. Only significant differences between pairs with the same aspect ratio or same unit cells per edge are shown.

In the case of compressive strength, both lattice types demonstrated a similar trend with the large-sized specimens where strength increased for decreasing aspect ratio. The stretch-dominated octet truss appeared again less affected by size effects (**Figure 6.13a**), with only the low aspect ratio ($\zeta=0.5$) specimen exhibiting slightly (6%) smaller strength when built using 3 unit cells along the vertical/loading direction compared to the bulk specimen (10 unit cells). In contrast, the bend-dominated BCC exhibited clear signs of size effects with specimens consisting of 3 unit cells demonstrating lower strength than the larger specimens of the same aspect ratio (**Figure 6.13b**).

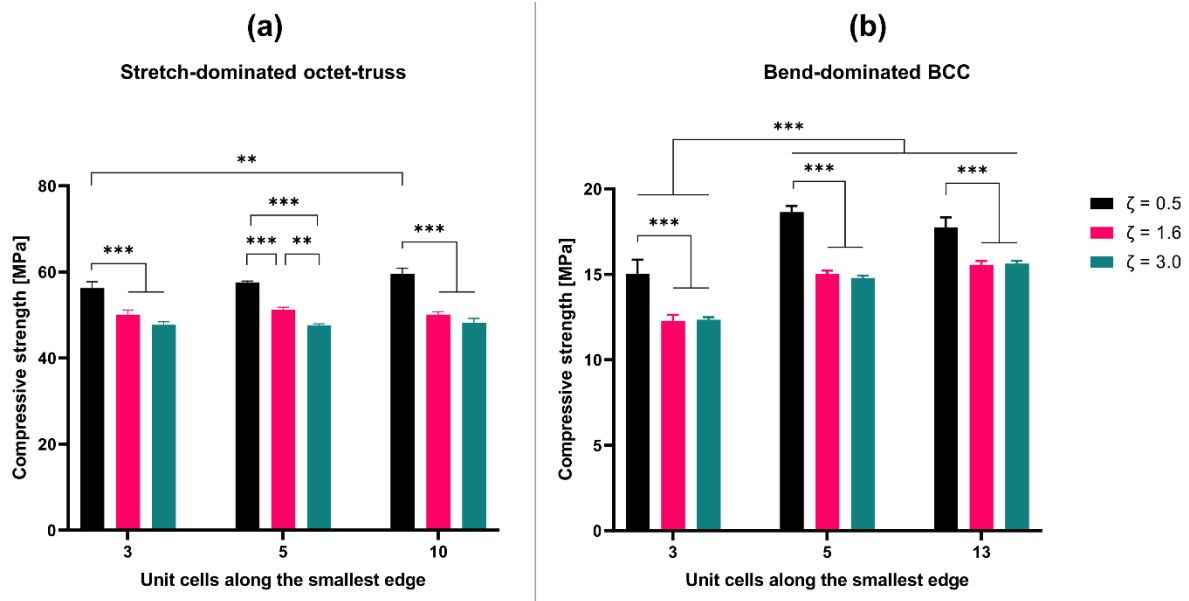


Figure 6.13. Effect of aspect ratio and specimen size on the compressive strength of octet-truss and BCC lattices. Statistical results are provided in Tables A.22 and A.23. Only significant differences between pairs with the same aspect ratio or same unit cells per edge are shown.

6.5. Results interpretation

The most important finding of this study is that the aspect ratio and topology of a lattice structure test specimen affect the measured mechanical properties and failure mechanisms. Increasing aspect ratio increased the measured modulus for stretch-dominated topologies but decreased the modulus for bend-dominated topologies. To a lesser extent, the aspect ratio also affected a specimen's measured strength, which decreased as the aspect ratio increased for both stretch and bend-dominated topologies. The reason for these findings is the number and location of strain shear bands that form within the specimen under load which depends on (a) the aspect ratio and (b) whether the specimen's topology is stretch or bend-dominated. These findings are important because many engineering components necessarily have aspect ratios outside the bounds of standards such as ISO 13314 and ASTM D1621. In these cases, it is critical to understand how properties and failure mechanisms of the in-situ component may deviate from the standardised test specimen geometry.

6.5.1. The effect of aspect ratio in large-sized specimens

For the bend-dominated BCC structures, failure patterns and strain accumulation captured through DIC highly align with previous experimental (319,344) and numeral models (149). Numerical studies on sandwich BCC lattices of different aspect ratio have shown that the structure's macro-stiffness is affected by local stiffness gradients due to the confined cells' deformation (149). In this study, the boundaries of the lattices were free and the stiffening of BCC lattices with decreasing aspect ratio was linked to end-induced stress concentrations, caused by the collapse of cells near the platens which generated high friction. This becomes more pronounced with increasing cross-sectional area (139). This only applied to BCC specimens with aspect ratio < 1 . BCC specimens with larger aspect ratio were not disrupted by end-effects or buckling and the measured stiffness remained constant.

For the stretch-dominated stochastic structures, there was no stiffening of the specimen at smaller aspect ratios. This was because the cells near the platens did not collapse since their nodes are more

tightly interconnected and the specimen behaves more like an indeterminate frame structure (95). However, the stretch-dominated structures exhibited stiffening at higher aspect ratios. This was considered to be caused by the formation of incremental distortion resulting in localised deformation bands well before the collapse point – this has been observed previously for foams (83) and in numerical models for lattices (151). The apparent increase in stiffness may have then been due to a larger portion of the specimen height remaining intact after the cells' initial distortion in the quasi-elastic region, making the structure more resistant to further deformation.

By testing two extra topologies: a bend-dominated stochastic structure and a stretch-dominated octet-truss structure, it was verified that the above stiffening mechanisms as well as the macroscopic failure modes observed were not topology-dependent but rather highly associated with the intrinsic micro-deformation mode of each structure (bend-dominated versus stretch-dominated). Additionally, the experimental observations regarding the failure mechanism of the BCC lattices with respect to different aspect ratios were found to be consistent at three distinct relative densities: 6%, 10% and 20%. The stiffening mechanism of the BCC lattice with decreasing aspect ratio was also verified for a relative density of 10%, but not in the case of 6% relative density. Thus, it seems that the effect of aspect ratio may attenuate for lattices with very low relative density.

Yield and compressive strength were found to be less impacted by aspect ratio, however, in all cases, their measured values increased at the smallest aspect ratios. This was more apparent for the BCC and octet-truss lattices which exhibited higher energy absorbance and plasticity, respectively. These observations for the octet-truss lattices greatly align with previous findings for quasi-brittle materials, such as metallic glasses, that also fail through shear band formation. From a thermo-mechanical perspective, higher post-yield softening (stress-drop) and lower plasticity have been linked to the larger elastic energy release for larger height or aspect ratio (345,346). This causes shear bands in high aspect ratio specimens to propagate rapidly leading to abrupt failure; however, in low aspect ratio specimens, multiple dispersed shear bands propagate slowly and do not connect to form a catastrophic crack. The latter may not have made all low ζ lattices stiffer, but it made lattices able to withstand larger compression forces prior to failure.

This study verified that lattices' mechanical properties are constant for aspect ratio between 1 and 2 as suggested by international testing standards, however they can vastly differ outside this region. The different trends of convergence in stiffness, strength and energy absorption for different lattice types support the notion that extended mechanical characterisation should be done prior to designing parts whose geometry falls outside this aspect ratio.

6.5.2. The effect of aspect ratio in small-sized specimens

A triple effect of aspect ratio, topology and size was investigated by comparing specimens built with 3, 5, and 10 unit cells across the smallest edge. In this study, two periodic topologies (BCC and octet-truss) were used to better control the arrangement of unit cells.

In line with previous research on metallic foams (135) and lattices (347), both lattice types encountered size effects where mechanical property values appeared decreased with decreasing size only when 3 unit cells were used. Compressive strength increased with decreasing aspect ratio in both topologies and across all specimen sizes, following the same trend as large-sized specimens. However, different stiffening mechanisms were observed for each topology.

The bend-dominated BCC lattice was the one with the most pronounced influence due to decreasing size. Strength and stiffness were found to decrease from the "bulk" values when less than 5 unit cells were used while employing a fixed aspect ratio, as previously seen in the bend-dominated Gyroid lattice

(347). However, the stiffening mechanism at the smallest-sized specimens reversed and stiffness appeared to increase with increasing aspect ratio. By taking into consideration that the high aspect ratio ($\zeta=3$) specimens exhibited similar stiffness across all sizes and the macroscopic failure pattern was consistent for fixed aspect ratio across all sizes, it can be inferred that BCC is highly sensitive to the number of cells stacked in the loading direction as specimen size decreases under a critical value.

The octet-truss specimens appeared unaffected by size effects even when designed with 3 unit cells along the shortest edge. The octet-truss retained the same strengthening and stiffening mechanisms with increasing aspect ratio as the large-sized specimens. Only the specimen with 5 unit cells along the Z-axis and $\zeta=0.5$ exhibited an unexpectedly high stiffness. Although this irregularity cannot be explained with the currently available data, it can be inferred that size and edge effects act synergistically towards a stiffening mechanism in the low aspect ratio.

These apparent differences between the BCC and the octet-truss are tightly associated with edge effects. Nodes at the free edges of BCC coupons may exhibit low connectivity ($Z=2$ or $Z=4$) or even consist of free-hanging struts ($Z=1$) depending on the neighbourhood of each unit cell. Free-hanging struts do not contribute to the load distribution, making the bend-dominated structure more prone to softening due to size effects. In contrast, nodes at the free edges of the octet-truss coupons are always interconnected with Z between 3 and 6 depending on the neighbourhood of each unit cell. This constitutes the outer nodes of the stretch-dominated lattices capable of carrying load and contributing to deformation resistance similar to the nodes at the core of the lattice.

6.5.3. Addressing challenges in mechanical testing of AM lattices

Variance in measured mechanical properties of cellular materials can be a product of manufacturing repeatability, specimen preparation, and testing protocols (83). Several factors were considered in this regard. Firstly, specimens of the same topology were fabricated in the same batches and in the same direction to minimise the variance associated with the additive manufacturing process. Hence, only the properties of specimens with the same topology were statistically assessed. Secondly, the periodic lattices were designed with cubic unit cells so that no aspect ratio ($\zeta \neq 1$) would be favoured due to the unit cell shape (130). Lastly, lattices were not printed attached to solid substrates/caps (83) to avoid constraining struts' deformation.

Typical forms of structural defects associated with powder bed fusion were also evident in the lattice specimens tested here. Waviness and deviation of strut thickness with respect to build angle have previously been shown to affect the failure mode and measured mechanical properties of lattice structures (348). Micro-CT analysis was used to assess the strut morphology across different lattice topologies. A limitation of this analysis was the use of a scan resolution (i.e., voxel size) comparable to the powder particle size, which also reflects typical forms of surface roughness (on the order of 10s of μm). Nevertheless, strut thickness was found to be highly consistent ($\approx 230 \mu\text{m}$) across different lattice topologies. Additionally, the arithmetic mean roughness (S_a) showed similar magnitude and trend, with values increasing for lower strut angles across all imaged specimens (**Figure A.2**). Although these data do not allow explicit characterisation of strut morphology, they suggest that the lattice fabrication process was consistent, regardless of strut angle, strut length, batch, or lattice topology.

Additionally, enclosed porosity is also known to affect the elastic properties of additively manufactured parts (349). While this may be primarily apparent to solid parts, lattices in this study were fabricated with relatively thin struts using low laser power (50 W) leading to near zero enclosed porosity as previously found in (89,349).

Overall, the coefficient of variance of measured values was between 1% and 10% which is in line with foreseen ranges for measured stiffness and strength in foams and lattices (83), demonstrating high consistency in the testing procedure.

Different-sized specimens were fabricated by stacking different numbers of unit cells across the smallest edge of periodic lattices for different aspect ratios. Based on previous studies (133,135,136), 3 and 5 unit cells were used to form the edges of small-sized specimens and at least 10 unit cells were used to form the edges of large-sized specimens.

The first part of the study aimed to isolate the effect of aspect ratio in adequately large specimens and results collectively advocate for its success. Both the Z8 stochastic and the BCC lattices achieved converged mechanical properties regardless of the size of the tested specimens. In addition, lattices of the same topology demonstrated similar failure patterns for fixed aspect ratios. A minimum of 10 unit cells were used to form the edges in the periodic lattices and the average strut distance in all lattices was more than 10 times higher than the specimens' smallest edge. All the above inform that specimens were large enough to capture "bulk" material properties with minimal influence by structural defects.

7. The effect of design parameters of stochastic lattices on osteogenesis

7.1. The effect of connectivity and strut density within stochastic lattices

7.1.1. Scaffolds characteristics

The structural and mechanical characteristics of the lattice designs are summarised in **Table 7.1**. High connectivity with high strut density (Z8-d7) led to high mechanical properties and low porosity, the low-low (Z4-d3) combination led to low mechanical properties and high porosity, and the two high-low combinations (Z4-d7 and Z8-d3) resulted in similar porosity and mechanical properties. For all samples the porosity fell within the range associated with trabecular bone (typically 70% to 90%) (19). Increasing Z or d resulted in less porous structures with smaller pores and higher surface area. The high connectivity structures (Z8-d3 and Z8-d7) exhibited the highest surface area which can be attributed to their longer struts (40% increased strut length for fixed strut density) and bulgier nodes (the Z8 nodes had ~2.5 times higher surface area than the Z4 nodes, see **Figure 7.1**).

Table 7.1. Characteristics of scaffolds with different Z and d, but fixed $t=230\ \mu\text{m}$.				
<i>Table reproduced under CC BY license from (121).</i>				
Properties and feature sizes	Lattice design			
	Z4 – d3	Z4 – d7	Z8 – d3	Z8 – d7
Porosity [%] (n=30)	91.6 $\pm$ 0.1	86.4 $\pm$ 0.4	87.9 $\pm$ 0.3	81.3 $\pm$ 0.5
Node density [nodes/mm ³]	1.6	3.4	0.7	1.7
Degree of Anisotropy	1.10	1.05	1.08	1.12
Strut distance [μm] *	1040 $\pm$ 310	750 $\pm$ 210	880 $\pm$ 370	680 $\pm$ 270
Strut length [μm] *	940 $\pm$ 220	720 $\pm$ 160	1320 $\pm$ 230	990 $\pm$ 180
Surface area [mm ²]	1354.9	1869.0	2108.9	2392.3
Surface area to volume ratio [mm ⁻¹]	1.868	2.728	3.141	3.587

* Values rounded to the nearest 10.

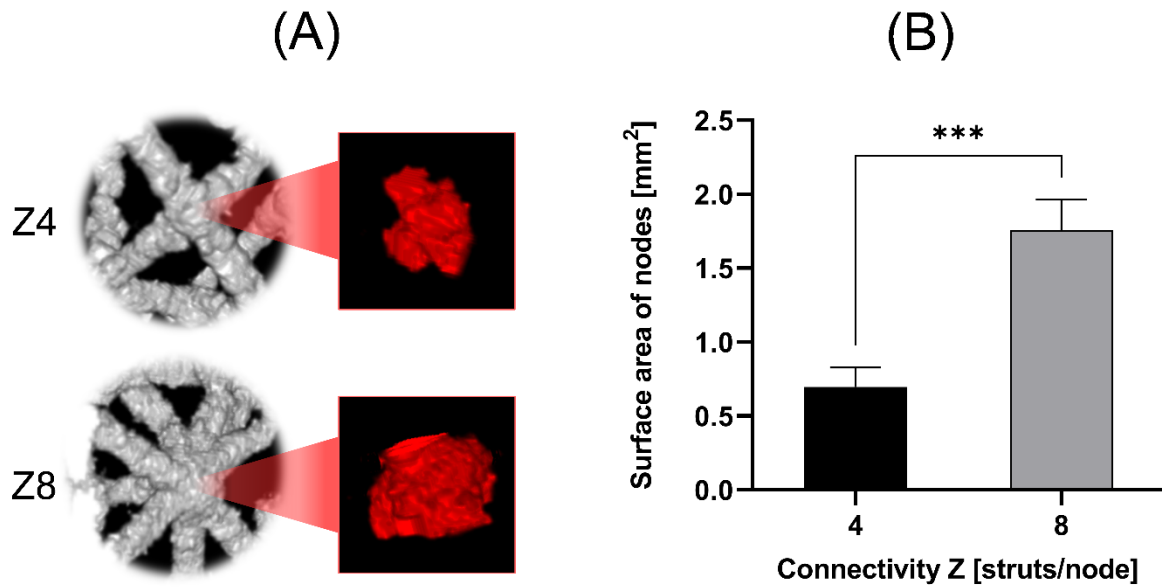


Figure 7.1. Comparison of surface area of nodes with 4 and 8 strut connections. Nodes were isolated from the micro-CT scans of scaffolds as shown in (A). The surface area of nodes with connectivity 8 was significantly higher ($n=5$, $p<0.001$) than the surface of nodes with connectivity 4 as shown in (B). Figure reproduced under CC BY license from (121).

The distance between adjacent struts within the structures was measured through micro-CT scans. This measurement informs about the size of voids within the stochastic structures and serves as an estimate for the pore size as defined in periodic structures. Valuable insights regarding stochastic topologies can be inferred by examining the distribution of strut distances for different connectivity levels Z and strut densities d (**Figure 7.2**).

Pore spacing (i.e., strut distance) demonstrated a narrower distribution in low-connectivity ($Z=4$) scaffolds, whereas high-connectivity ($Z=8$) scaffolds showed greater variance. This difference arises because higher connectivity increases spatial tessellation, introducing multiple corners around the nodes and multiple pores inside the structure. A narrow peak, as seen in the Z4 structures, suggests a more uniform lattice with more evenly spaced struts. On the contrary, lack of a distinct peak, as seen in high-connectivity structures, suggests a heterogeneous structure with a mix of large and small pores.

Additionally, high-strut-density structures ($d=7$) exhibited a more confined range of pore sizes, ensuring that strut distances remained below 1300 μm . In contrast, low-strut-density structures showed larger voids, which could potentially hinder cell communication.

Nevertheless, structures followed a pseudo-random architecture with similar strut orientation in space leading to similar degrees of anisotropy (115). In addition, structures employed struts in all directions resulting in a degree of anisotropy near 1 (i.e., isotropic material). While trabecular bone is a highly anisotropic material where the degree of anisotropy ranges from 1.1 to ≈ 5 (120), having isotropic properties in a lattice is important from a design perspective as it allows better prediction of lattice behaviour *in situ*.

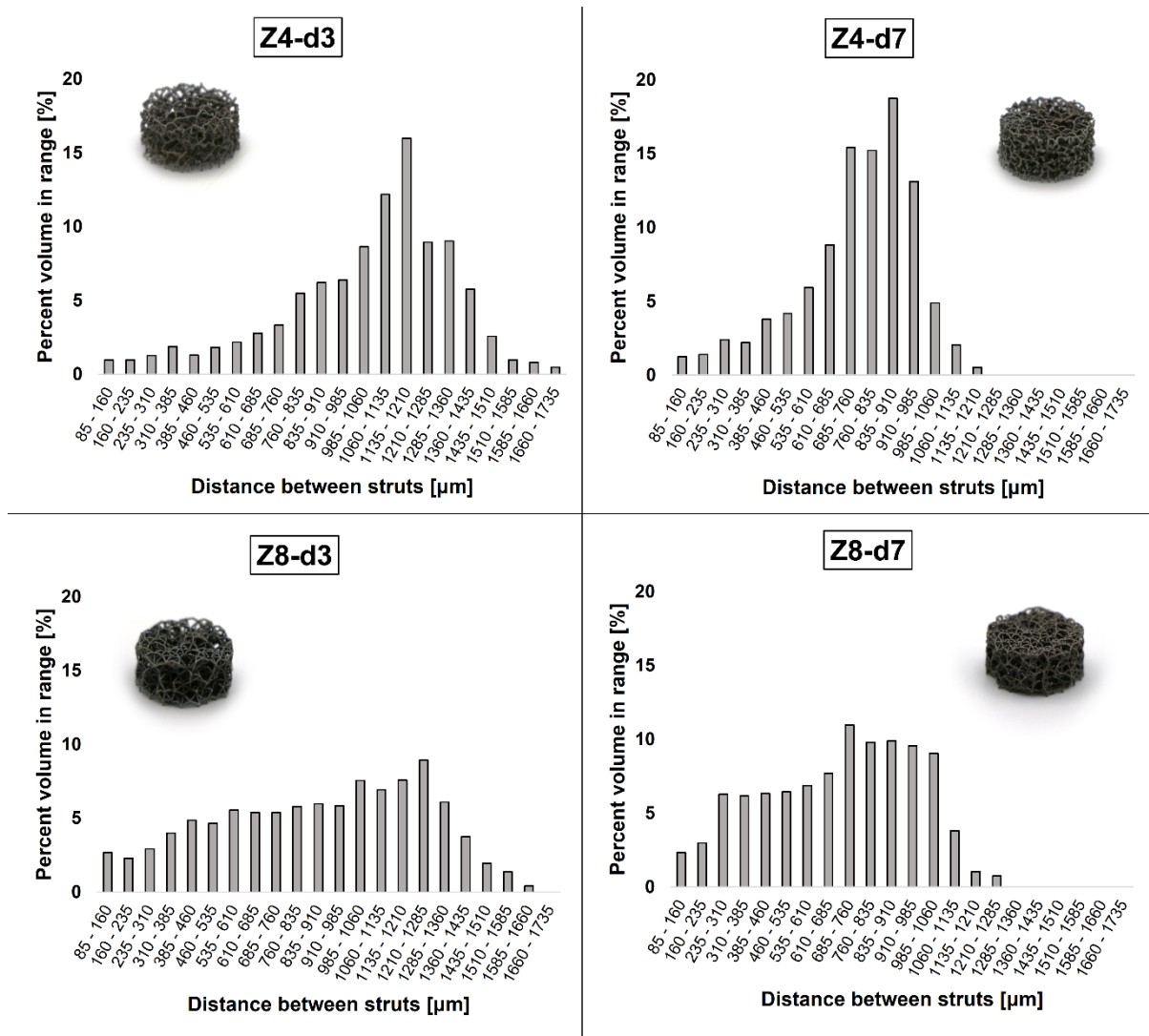


Figure 7.2. Distribution of distances between adjacent struts within stochastic structures. *Figure reproduced under CC BY license from (121).*

Scaffolds exhibited the same defects and surface roughness (**Figure 7.3A**) as discussed in the previous chapters. This time, surface roughness was quantified using individual struts and a digital microscope's 3D optical profilometer to highlight the effect of strut angle and upward/downward-facing surfaces. As shown in **Figure 7.3B**, the average roughness (R_a) was found to range between 5 and 30 μm . As seen in **Chapter 6** and **Figure A.2**, R_a values tend to increase with decreasing build angle. Furthermore, this analysis demonstrated that the downward-facing surface of struts is always rougher than the upward-facing surface due to higher heat accumulation. This is particularly apparent for struts with $\leq 30^\circ$ inclination due to excess cross formation at the downward-facing surface.

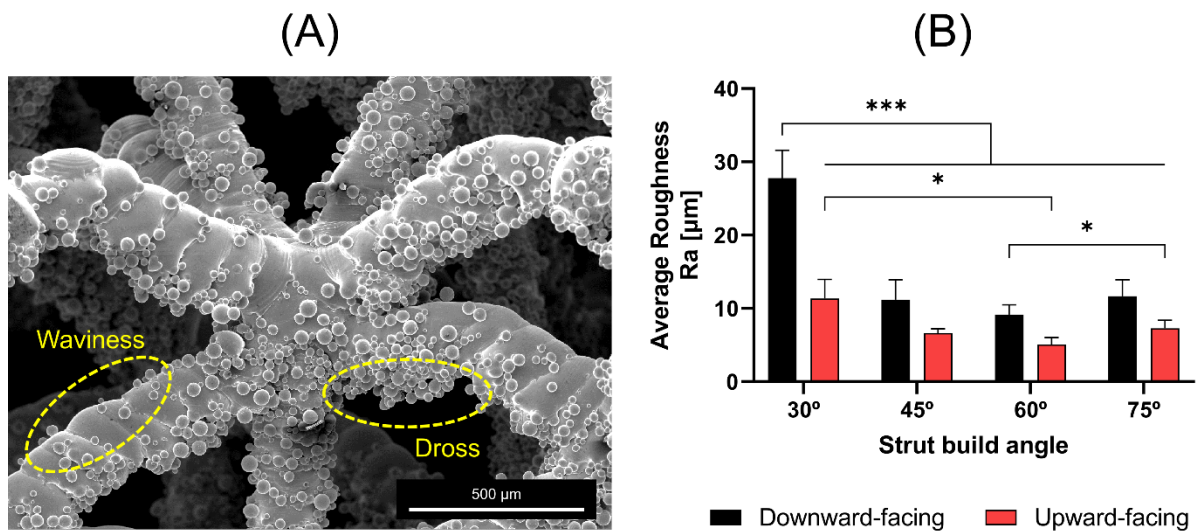


Figure 7.3. Quantification of surface roughness on struts with 230 μm thickness. (A) SEM images displaying typical forms of roughness, such as semi-sintered particles, waviness, and dross due to overheating. (B) Arithmetic average roughness (R_a) of the upward and downward-facing surfaces of struts built at different angles. Measurements were obtained using a 3D optical profilometer on a digital microscope, with roughness evaluated along a 75-μm profile line at the midsection of each strut. For each build angle, three unique struts were analysed, with measurements taken on both the upward and downward-facing surfaces. Each column represents the mean $\pm$ standard deviation. Figure reproduced under CC BY license from (121).

7.1.2. Cell adhesion and proliferation

All samples achieved similar metabolic activity in the first 24 hours (**Figure 7.4A**), suggesting that cell seeding and cell adhesion were equivalent among all scaffold designs. Cell seeding efficiency was estimated to be approximately 85% for all scaffolds.

To assess cell growth trends over time, fluorescence intensity values were measured every three days, with all measurements from day 3 onward normalized to day 0 (**Figure 7.4A**). Metabolic activity increased on all scaffold designs, aligning with expected cell proliferation patterns. The proliferation rate varied significantly depending on the culture day ($p < 0.001$), increasing from day 0 to day 6 before plateauing. It also differed among scaffold designs: Z4-d3 scaffolds exhibited a 20-161% higher metabolic activity increase compared to other scaffold designs ($p < 0.01$).

This pattern is characteristic of MC3T3 cell growth and may indicate the transition from proliferation to differentiation and ECM formation, with no signs of prevalent cytotoxicity (330,350).

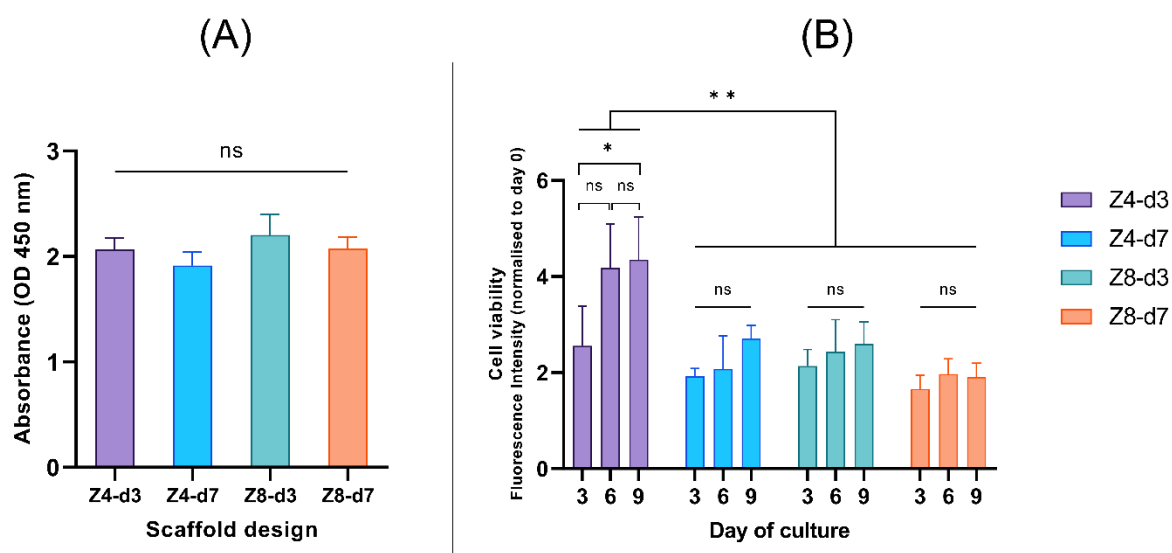


Figure 7.4. Cellular metabolic activity in scaffolds with different Z and d . (A) The high-sensitivity CCK8 kit was used to compare cell adhesion in the first 24 hours. Reproduced under CC BY license from (121). (B) Cell viability was used to monitor the proliferation rate of cells over the first 9 days. Each column corresponds to the mean value of $n=3$. ANOVA results are provided in Tables A.24 and A.25 in the Appendix. The main effect of day ($p<0.001$) is not highlighted.

7.1.3. Cell viability observation

Representative fluorescent images of the live/dead assay at four time points were displayed in **Figure 7.5** with similar characteristics seen at all designs. The pervasiveness of live cells over dead cells was apparent in all scaffolds, suggesting high cell viability. Observation of the top and bottom surfaces of the scaffolds as well as their periphery demonstrated that cells successfully migrated inside the lattice structures of the scaffolds. Distinct cells spread along struts were seen on day 7, while scattered small green regions of cell clusters were seen after day 14 along struts and around the nodes. On days 21 and 28, larger green regions of more accumulated cell clusters were stained. These groups of cells were closer to each other covering larger areas of the scaffold's network, indicative of new ECM and tissue formation.

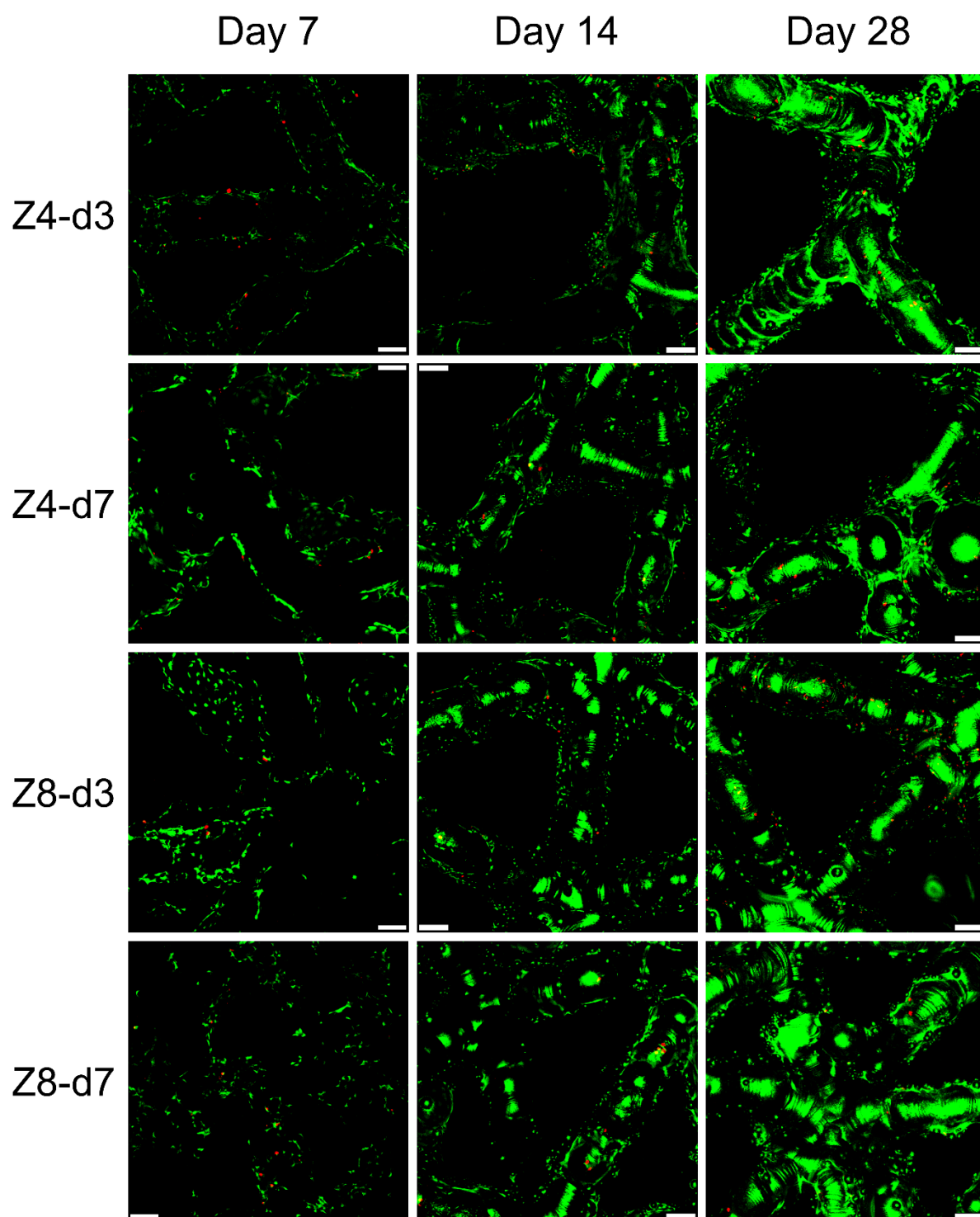


Figure 7.5. Fluorescence images showing live/dead staining on scaffolds with different Z and d . Live and dead cells are stained green and red, respectively. Images were taken using 10 $\times$ objective lens. Scale bars correspond to 100 μm . Subfigures for Z4-d3 and Z8-d3 reproduced under CC BY license from (121).

7.1.4. Morphological evaluation under SEM

SEM imaging was used to highlight key aspects of cellular proliferation and matrix formation inside porous scaffolds. Individual cells along struts and formed ECM at the corners (**Figure 7.6A**) were seen on day 7. In the early stages of cell culture (days 7 and 14), cells were spread and anchored around the semi-sintered powder particles. Formed ECM bridging the corners of nodes was seen after day 14 (**Figure 7.6B**) with concave angles facilitating the phenomenon. ECM formation progressed rapidly up to day 21 (**Figure 7.6C**) and appeared to stabilise at day 28 (examples of low and high connectivity structures are shown in **Figure 7.7**). Still, the thickening of the ECM was distinguishable with the progress of culturing weeks (**Figure 7.8**).

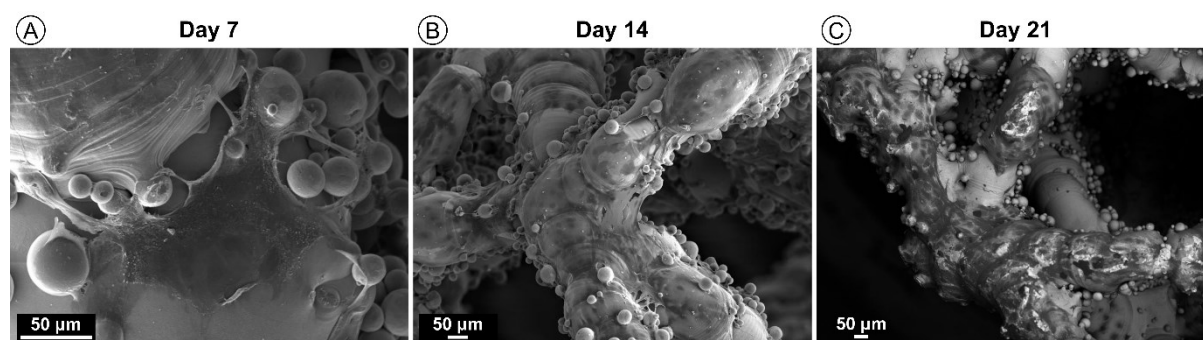


Figure 7.6. Representative SEM images on days 7, 14, and 21. All specimens correspond to lattices with Z4. Subfigures A and B reproduced under CC BY license from (121). Backscatter images were taken on day 21 to distinguish the grown ECM from the titanium surface.

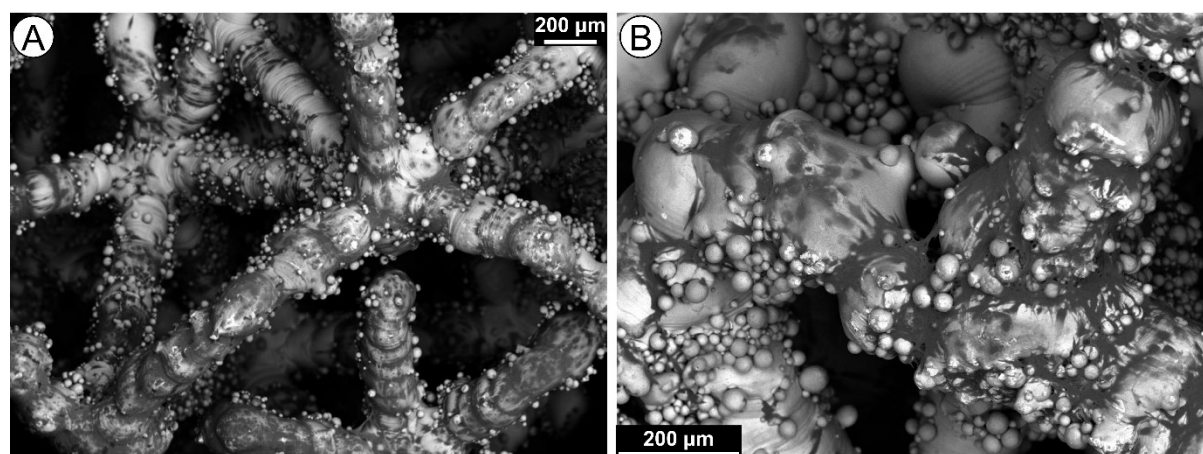


Figure 7.7. Backscatter SEM images on day 28 from scaffolds with different Z. A lattice with Z8 is shown in (A) and a lattice with Z4 in (B). Subfigure (B) reproduced under CC BY license from (121).

The bridging of the corners was not as pronounced as expected, instead, ECM was seen to primarily wrap along the struts' length. This can be attributed to the relatively long struts and large pores employed by all scaffold designs. In addition, cell adhesion appeared to be favoured by the rough surface of the additively manufactured struts (cells clustered around the semi-sintered particles) as well as the developed film due to the alkali treatment (**Figure A.2** in the **Appendix**). SEM images, here, demonstrated that this thin micro-porous coating (oxide layer) provided a cell-friendly substrate topography aiding the initial cell adhesion, and later the spread of ECM inside the scaffolds (**Figure 7.8**).

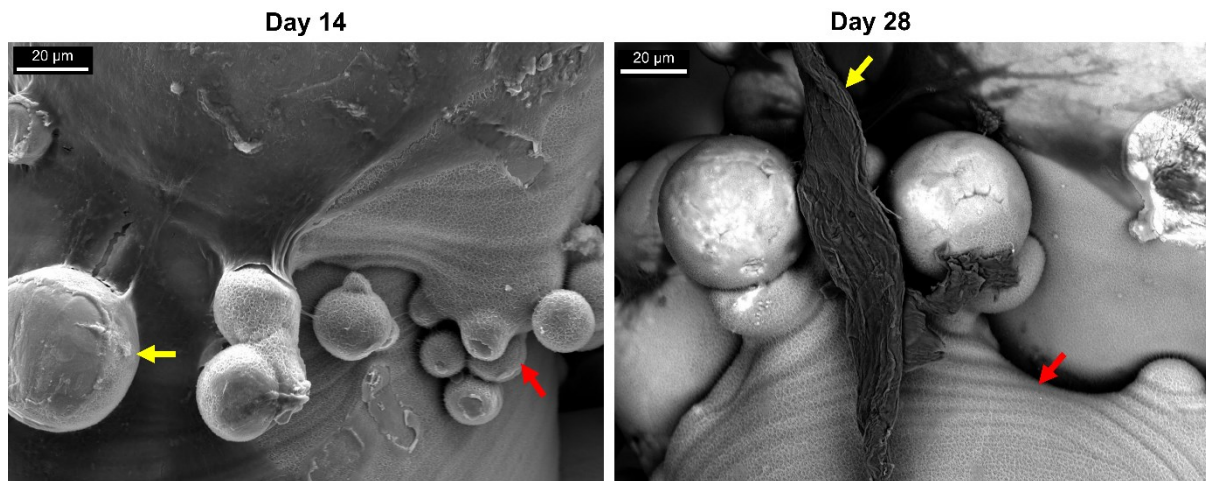


Figure 7.8. SEM images showing ECM anchoring and growth on the oxide layer. Yellow arrows indicate formed ECM and red arrows indicate the porous film due to NaOH treatment. Figures reproduced under CC BY license from (121).

7.1.5. Collagen content

Macroscopic images demonstrated that ECM rich in collagen content was formed deep inside all scaffolds and covered most of their surface area (**Figure 7.9a**). Measurements of the absorbance of dissolved dye are shown in **Figure 7.9b**. At day 7, both the higher strut density designs (Z8-d7 and Z4-d7) had more than double the collagen content compared to the low-low combination (Z4-d3, both $p < 0.05$). Collagen content increased by 87-265% for all samples from day 7 to day 28 ($p < 0.001$) indicating the progressive growth and maturation of the ECM. By day 28, the densest scaffold (Z8-d7) had ~30% more collagen accumulation than the other designs ($P < 0.05$).

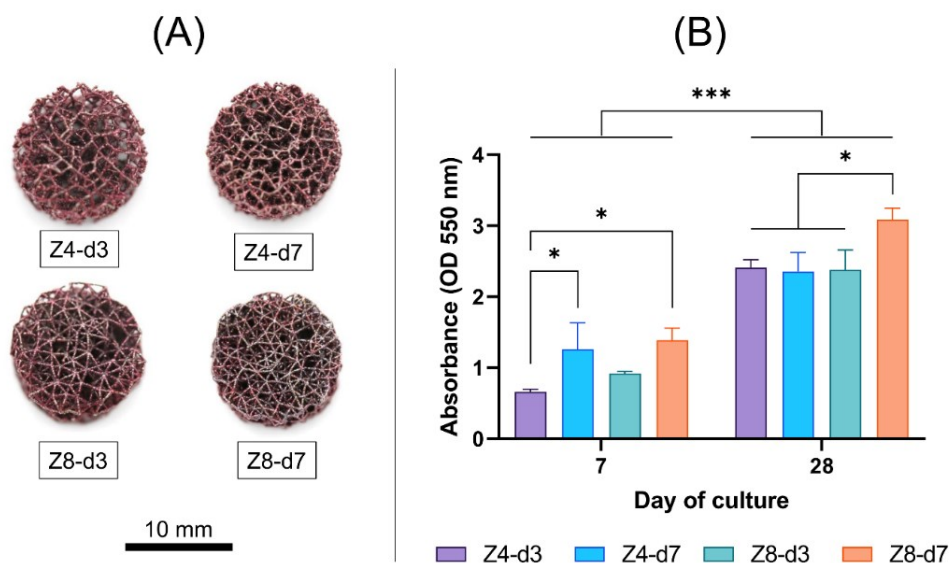


Figure 7.9. Picrosirius red staining on scaffolds with different Z and d. Microscopic images of the stained scaffolds are shown in (A). The bound dye was removed from scaffolds and collagen content was quantified by measuring the optical density. Each column corresponds to the mean value of $n=3$. ANOVA results are provided in Table A.26 in the Appendix. Figure reproduced under CC BY license from (121).

7.1.6. Mineralised matrix labelling

As expected, no fluorescence was detected in any scaffold at day 7 as pre-osteoblasts were still in their proliferative phase. Minor green stained dots were observed on day 14 (**Figure 7.10**) indicating the initiation of minerals deposition. At days 21 and 28 abundant green regions covered larger areas of the scaffolds' surface (**Figure 7.10**), indicating gradual mineralisation and bone tissue formation.

At the end of the culture, extended mineralised zones were found at multiple regions (corners) around nodes mostly in high connectivity (Z8) scaffolds (**Figure 7.11**). This differed from the low connectivity (Z4) scaffolds where mineralised ECM was observed on struts' surface with limited bridging of the corners (**Figure 7.11**).

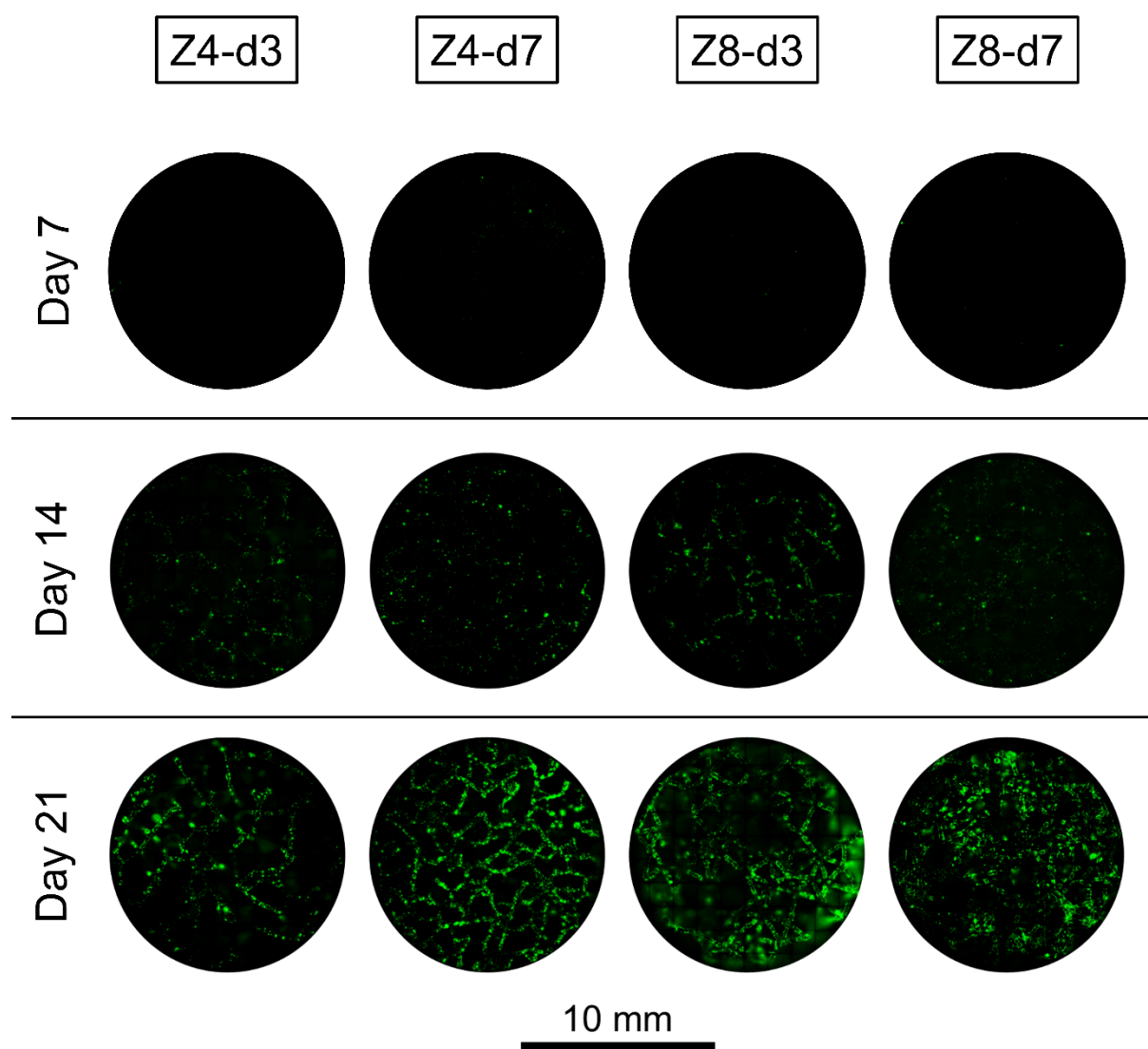


Figure 7.10. Macroscopic fluorescence images of calcein staining on days 7, 14 and 21. Green areas indicate calcified matrix. The absence of green areas at day 7 suggests a lack of calcium deposition and confirms the absence of autofluorescence from the titanium scaffolds. Subfigures reproduced under CC BY license from (121).

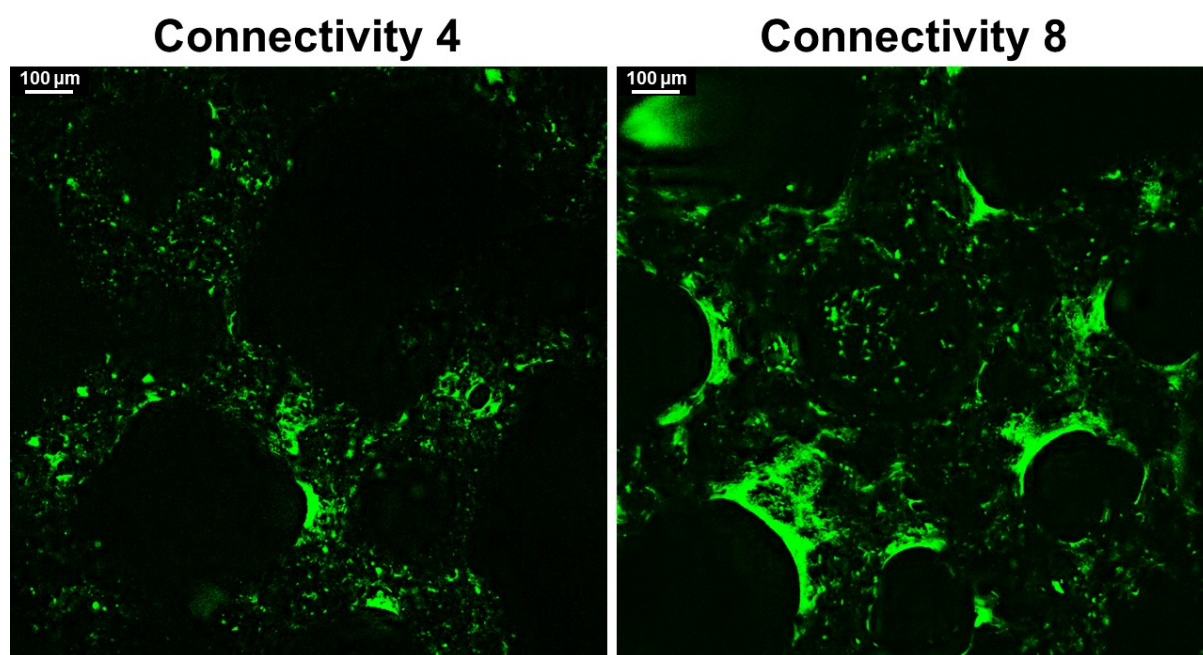


Figure 7.11. Magnified fluorescence images of calcein staining on day 28. Green areas indicate calcified matrix. Subfigures reproduced under CC BY license from (121).

7.1.7. Osteoblast maturation

Representative time-points (days 7, 14, 21 and 28) were chosen to compare the progress of pre-osteoblasts differentiation among designs. The ALP activity changed with the day of culture (**Figure 7.12**). Enzyme's activity only started to increase by day 14, where it increased by 314-607% for all designs between days 14 and 21 in all designs ($p < 0.05$), indicating the initiation of cell differentiation. After 28 days, only the strut density d3 continued to express 49-128% increased level of the enzyme's activity ($p < 0.05$), while the activity was similar for the d7 designs.

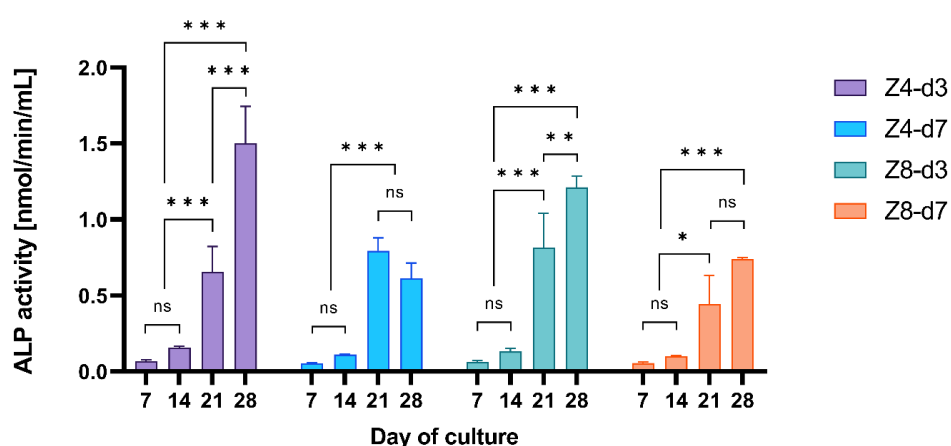


Figure 7.12. ALP activity in scaffolds with different Z and d. Measurements were taken using the supernatants in the culture media. Each column corresponds to the mean value of $n=3$. ANOVA results are provided in Table A.27 in the Appendix. Only differences within the sample design are shown.

The expression levels of collagen type-I, osteopontin and osteocalcin at day 7 and day 28 are shown in **Figure 7.13**. Differences between designs were only apparent at day 28 ($p < 0.05$), agreeing with the ALP finding that cell differentiation had not measurably initiated by day 7. Thus, the levels of these (primarily late-stage) osteogenic biomarkers had more than doubled by day 28 compared to day 7 ($p < 0.001$). More importantly, though, the different effect of each design parameter was observed depending on the biomarker.

Connectivity and strut density alone were found to influence the expression of Collagen Type-I ($p < 0.05$). For fixed strut density, high connectivity (Z8) scaffolds had higher expression of collagen. While for fixed connectivity, scaffolds with higher strut density (d7) scaffolds exhibited relatively upregulated levels of collagen compared to low strut density (d3) scaffolds.

Only connectivity had affected the expression level of osteopontin ($p < 0.05$) with high connectivity (Z8) scaffolds exhibiting $\sim 2\times$ more upregulated levels than low connectivity (Z4) scaffolds ($p < 0.001$). Lastly, connectivity (Z) also influenced the expression levels of Osteocalcin ($p < 0.05$), but it depended on the strut density. This can be perceived by the 112% increased levels of osteocalcin in the Z8 scaffolds compared to the Z4 scaffolds for the low strut density (d3).

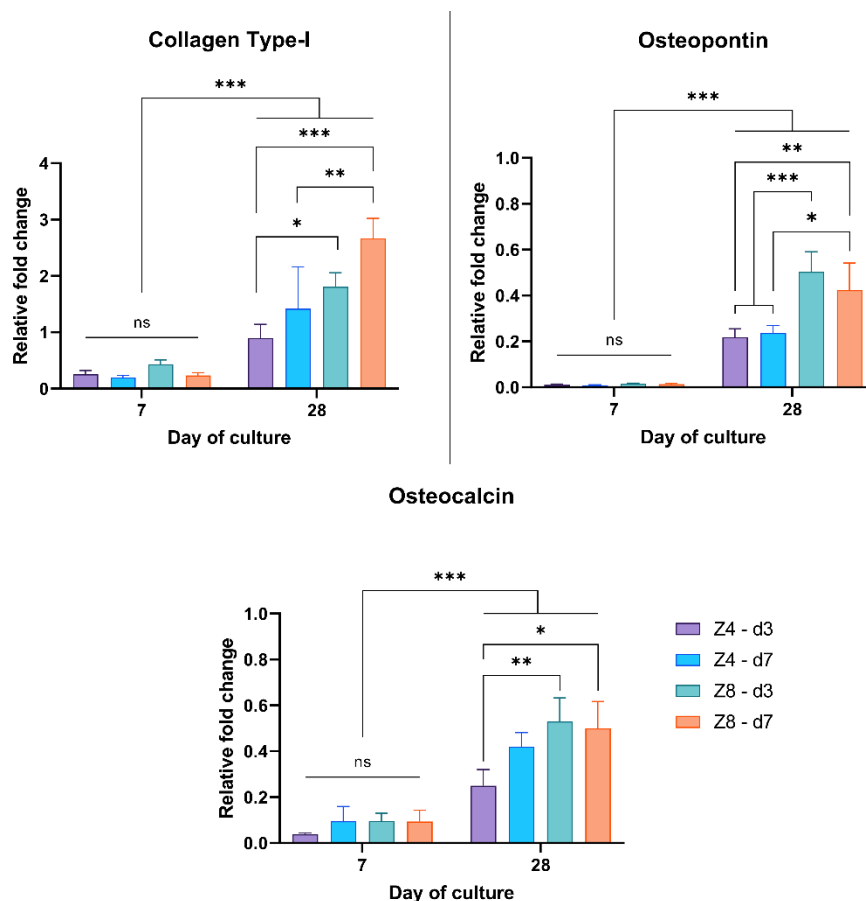


Figure 7.13. The expression levels of collagen type-I, osteopontin, and osteocalcin on days 7 and 28 in scaffolds with different Z and d. Measurements were taken using RT-qPCR and values were normalised to β -actin. Each column corresponds to the mean value of $n=3$. ANOVA results are provided in Tables A.28-A.30 in the Appendix. Subfigures reproduced under CC BY license from (121).

7.2. The effect of strut thickness within stochastic lattices

7.2.1. Scaffold characteristics

To isolate the effect of strut thickness on cellular behaviour, the same scaffold design (Z6-d3) was fabricated with two critically different strut thicknesses t (**Figure 7.14a**). The average strut thickness for the thin-strut scaffolds was found $169.0 \pm 4.5 \mu\text{m}$, while for the thick-strut scaffolds was found $302 \pm 13.4 \mu\text{m}$. In both cases, the consistency in strut size across different build angles was validated using μCT scans and StrutSurf (333) (**Figure 7.14b**). Using the same method, the average surface roughness (S_a) was found significantly higher ($p < 0.001$) in the thick struts ($9.7 \pm 1.4 \mu\text{m}$ in the thin-strut scaffolds and $14.9 \pm 3.5 \mu\text{m}$). The increase of roughness with increasing strut size should be associated with the $\times 2$ higher enthalpy and the $\times 4$ higher laser power used for the fabrication of thick struts (351). Interestingly though, the roughness-to-thickness ratio S_a/t was similar for the two strut sizes, 6% for the thin struts and 5% for the thick struts.

The morphological characteristics of the two scaffolds are summarised in **Table 7.2**. Increasing strut thickness resulted in a drastic reduction of scaffold porosity (-17%) and a drastic increase in stiffness ($\approx 3 \text{ GPa}$ more), with mean strut distance, however, being less affected. Increasing strut thickness also led to a higher (absolute) surface area. However, the surface area to structure volume ratio of the thin-strut scaffold was more than double that of the thick-strut scaffold.

Table 7.2. Characteristics of scaffolds fabricated with different strut sizes		
Properties and feature sizes	Nominal strut thickness	
	170	300
Porosity	91%	74%
Elastic modulus [MPa] (n=3)	468.1 ± 12.6	3455.5 ± 754.0
Strut distance [μm] *	1100 ± 450	900 ± 430
Strut length [μm] *	1600 ± 400	1600 ± 400
Surface area [mm^2]	530.2	777.9
Surface area to volume ratio [mm^{-1}]	24.84	12.22

* Values rounded to the nearest 10.

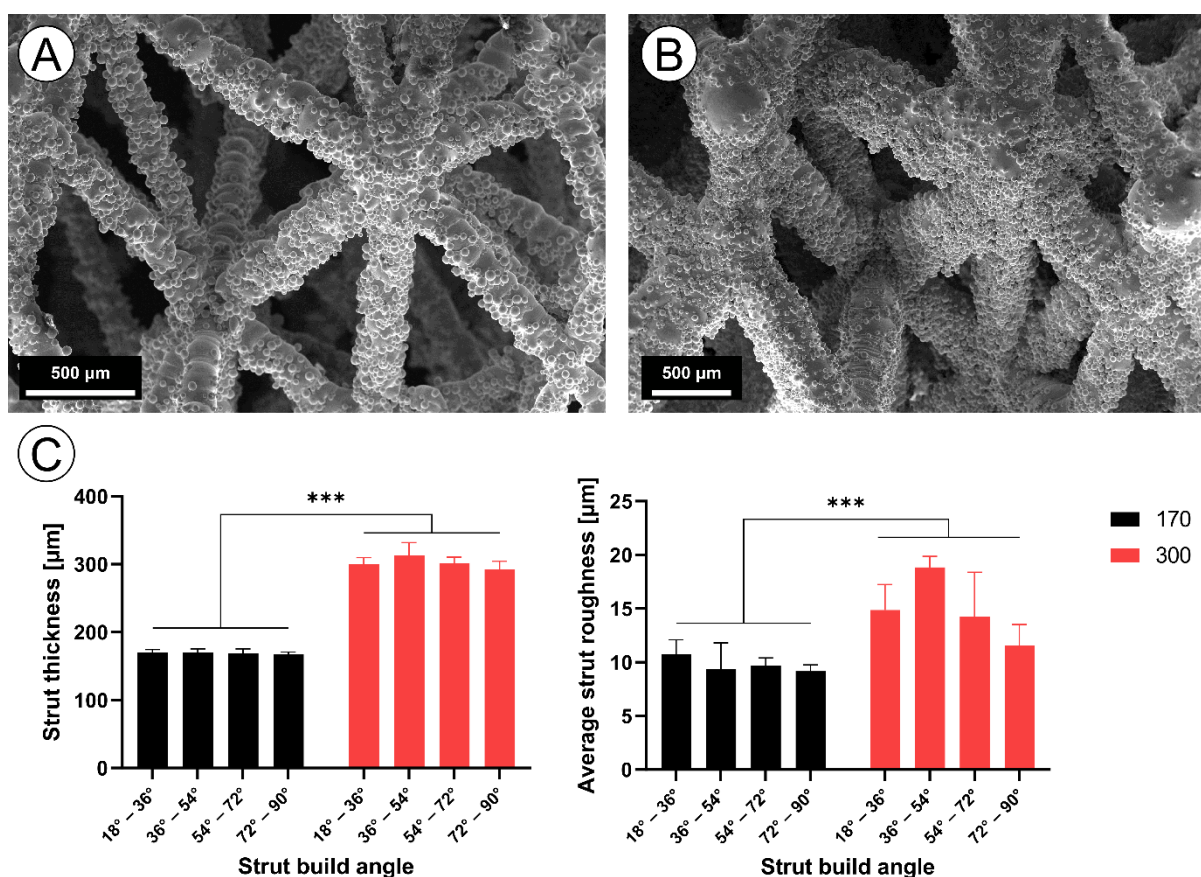


Figure 7.14. Imaging and morphological evaluation of scaffolds built with different strut sizes. Representative SEM image of (A) thin-strut and (B) thick-strut scaffold. (C) Micro-CT data were used to quantify strut thickness and average strut roughness (S_a) across different build angles by fitting a 3D cylinder around 20 randomly picked struts in each sample.

7.2.2. *In-vitro* cell response

Similarly to the previous experiment, cell metabolic activity (**Figure A.6** in the **Appendix**) was used as an estimate of cell growth and viability. Fluorescence intensity significantly increased on day 3 in both scaffold types compared to day 1 ($p < 0.001$ and $p < 0.05$ for the thin-strut and thick-strut scaffolds, respectively), which may indicate a proliferative phase. The comparison of fluorescence intensity at day 3, normalised to day 1 (**Figure 7.15a**), further suggested that the relative increase in metabolic activity was significantly higher in the thin-strut scaffold than in the thick-strut scaffold ($p < 0.01$).

Cell lysates were used to quantify key osteogenic biomarkers in each scaffold, which constitutes a more accurate method than using culture supernatants used before. ALP activity peaked at day 7 in both scaffolds and then decreased at day 14 (**Figure 7.15b**), indicating a quick mineralisation cycle (≈ 10 days) compared to what was observed in the previous experiment. Statistical analysis demonstrated that the thin-strut scaffolds induced significantly higher ALP expression ($p < 0.001$). This was in line with the measurements of the osteocalcin at day 14 (**Figure 7.15c**) which again were higher in the thin-strut scaffold ($p < 0.05$).

In contrast to the previous experiment, this one exhibited a notably shorter mineralisation cycle, with ALP values drastically decreasing by day 14. This is a common phenomenon that has been reported in other works using porous biomaterials (352,353), and should be attributed to the high seeding density used and is known to accelerate tissue formation and mineralisation (354,355). In detail, the previous experiment employed a seeding density of $\approx 1.5 \times 10^6$ cells/cm³, while this one $\approx 2.3 \times 10^6$ cells/cm³.

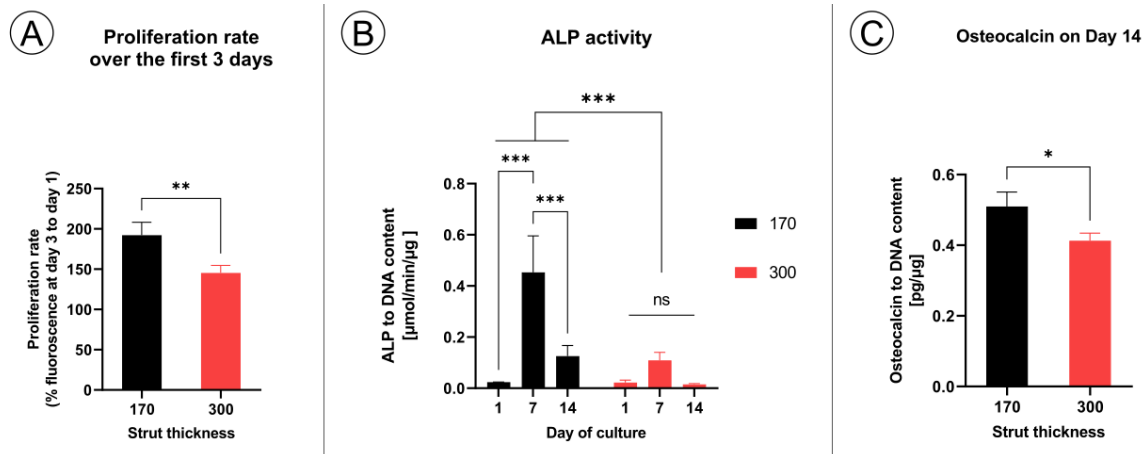


Figure 7.15. Evaluation of proliferation and maturation of MC3T3 within scaffolds with different strut thicknesses. (A) The proliferation rate in the first 3 days was quantified by normalising fluorescence at day 3 to the one at day 1 ($n=4$). For maturation, the intracellular levels of (B) ALP and (C) osteocalcin were measured using cell lysates and normalised to total DNA content ($n=3$ for both).

Confocal microscopy (Figure 7.16a) and SEM (Figure 7.16b) were used to visualise the progress of cell proliferation and matrix deposition in both scaffold types throughout these 21 days of culture. Both imaging methods made apparent the prevalence of cell number in the early days (days 1 and 11) and the extent of the formed matrix in the thin-strut scaffolds compared to the thick-strut scaffolds. Individual cells can be seen along the struts and around the nodes in the images from days 1 and 11. While cellular metabolic activity (Figure 7.15a) indicated that an equal number of cells adhered to both scaffolds, these images point out that thin struts make it easier for cells to migrate and meet other cells compared to large struts where cells have to travel a greater distance to meet neighbouring cells.

Last, one sample per strut size was used to stain the mineralised matrices. Two stains were applied on day 14 (Calcein shown in green) and day 21 (Alizarin Red shown in red). Representative images are shown in Figure 7.17. Although a visual method, the results suggest that mineralisation was higher in the thin-strut scaffolds with large green areas covering struts and bridging the nodes from day 14. Multiple red dots indicating further calcium crystallisation were also seen on day 21. On the contrary, the thick-strut scaffolds exhibited smaller green regions (both along the struts and around nodes) indicating a lower mineralisation rate. Distinct red regions were seen here too. An apparent difference between the two images is that mineralisation in the thin-strut scaffolds appeared to be more homogeneously spread within the scaffold, while a more heterogeneous distribution of mineralised matrix was seen in the thick-strut scaffold.

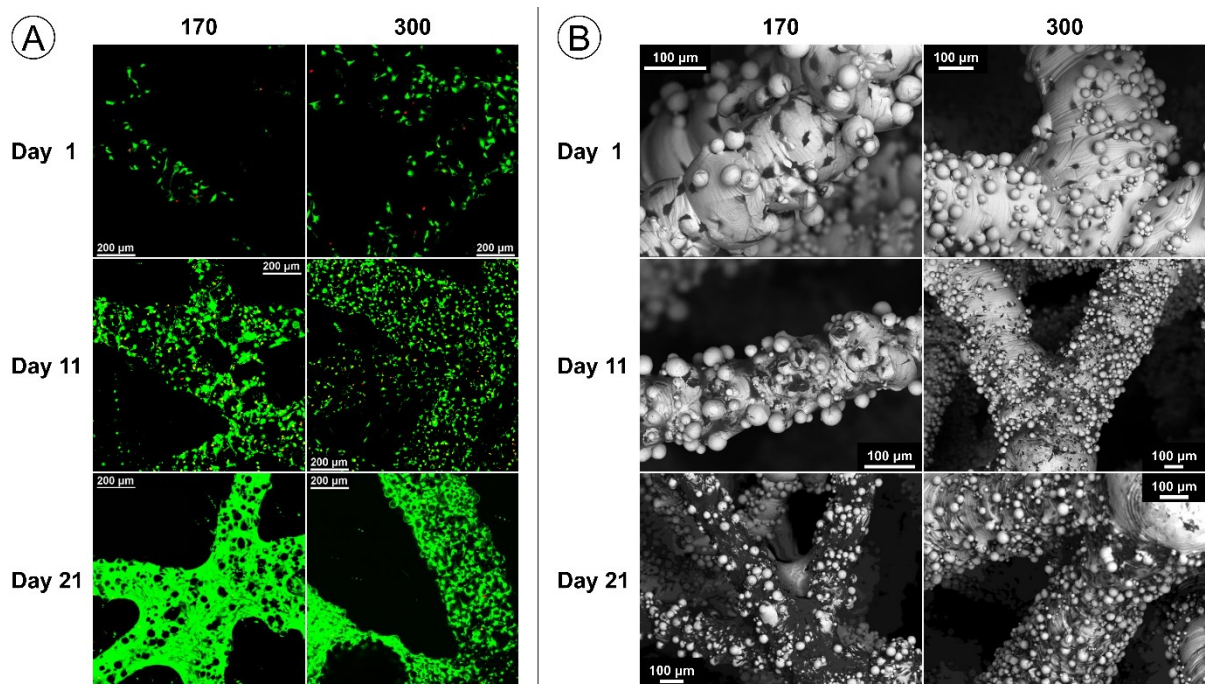


Figure 7.16. Imaging of cells and formed matrix in scaffolds with different strut thicknesses. (A) Live-dead imaging was used to confirm and monitor cell viability within scaffolds up to day 21. (B) The same samples were used in backscattered SEM.

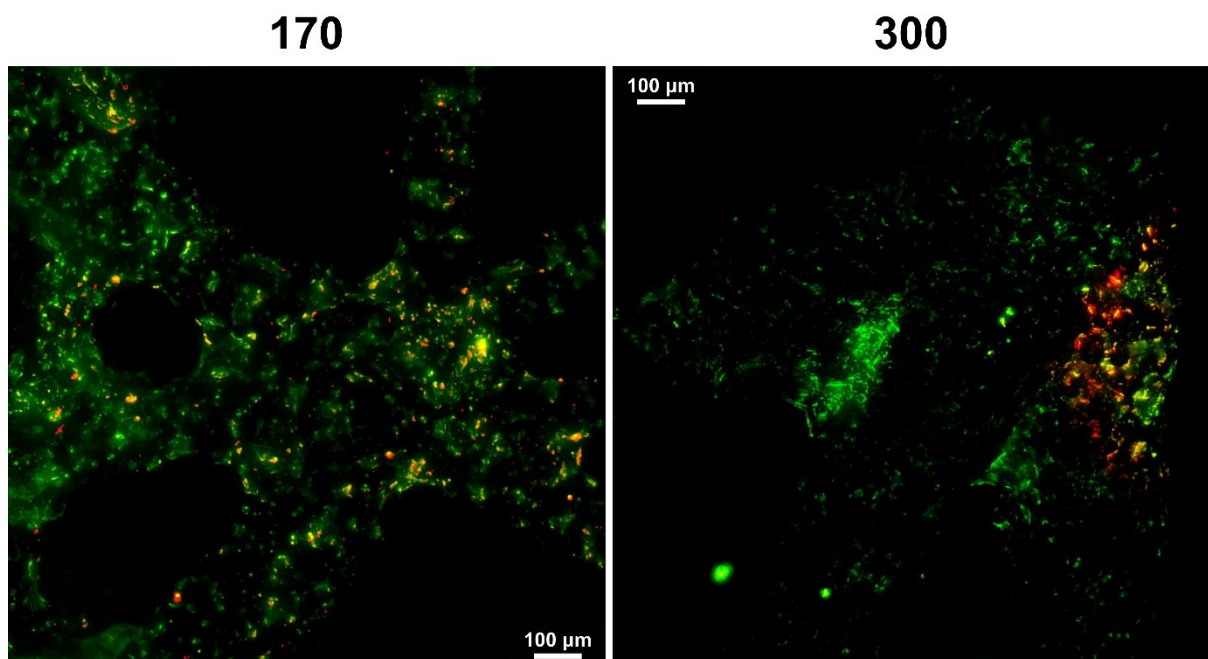


Figure 7.17. Progress of matrix mineralisation in scaffolds with different strut thicknesses. Calcein (shown in green) was added on day 14, and Alizarin Red (shown in red) was added on day 21.

7.3. Results interpretation

Nodal connectivity, strut density, and strut thickness can be tailored to generate stochastic lattice structures that match the mechanical properties of trabecular bone. This study demonstrated how these design parameters can also regulate the micro-architecture and biological performance of cell-seeded scaffolds. Increasing strut density leads to denser structures with smaller pores, while high connectivity, not only decreases porosity, but also results in tightly interconnected structures with increased surface area, numerous pores, and corners around nodes. Strut thickness is a structural parameter with a profound impact on mechanical properties, increasing its value results in lower porosity with a drastic increase in stiffness.

In this chapter, titanium scaffolds with different geometrical characteristics were evaluated for their performance on cell adhesion, cell proliferation, cell differentiation and their ability to form bone-like tissue. A collective consideration of the results can bring out the effect of each design parameter on osteogenesis and help adjust its values to generate stiffness-matched lattices with a micro-architecture that favours osteoblastic differentiation.

7.3.1. Connectivity versus strut density

The first part of this chapter aimed at evaluating the effect of connectivity and strut density using four scaffolds designed with two levels of connectivity, two levels of strut density, and a fixed strut thickness.

Cellular metabolic activity was used as an indirect metric to assess cell viability and estimate proliferation within scaffolds during the first nine days of culture. Metabolic activity levels at 24 hours suggested comparable initial cell attachment across all scaffold designs, despite differences in average strut distance (ranging from 680 to 1040 μm). This finding contrasts with previous studies which evaluated cell adhesion (i.e., cell seeding efficiency) on periodic scaffolds of similar pore sizes (500 to 1000 μm) (8,42). Specifically, in these studies, scaffolds with smaller pores demonstrated significantly higher cell attachment than scaffolds with larger pores, albeit scaffolds were made of the same material and pore geometry. Although cell adhesion on periodic scaffolds has been associated with the available surface area that cells can adhere to (357), in our case scaffolds with different surface areas demonstrated equivalent seeding efficiency and cell adhesion. This can be attributed to the lower fluid permeability of stochastic lattices compared to periodic lattices (358). The difference in permeability has been shown to originate from the increased tortuosity in stochastic micro-architectures (like the trabecular bone) compared to periodic ones (359). Ultimately, lower permeability and higher tortuosity result in lower velocity and impeded flow of the cell suspension during seeding which allows cells to attach to the scaffold's surface (257,360).

Regardless of the equal cell attachment on all scaffolds at day 1, the most porous scaffold (Z4-d3) exhibited the highest proliferation rate with cell number quadrupling the following days of culture, while less than a threefold increase was seen in the rest of the designs. These observations are in line with previous studies in periodic scaffolds with similar pore sizes (500 to 1000 μm) (257,356), where larger pores have been shown to induce higher cell proliferation. Nevertheless, colourimetric measurement of the bound Picrosirius Red indicated that the denser scaffold (Z8-d7) induced a significant amount of collagen within expanded areas of newly formed ECM compared to the rest of the designs. The contradiction between the prolonged cell proliferation in the most porous Z4-d3 compared to the enhanced ECM maturation in the densest Z8-d7 should be attributed to the combination of high connectivity, shorter strut distance and larger surface area of the Z8-d7. High porosity results in uneven cell distribution inside scaffolds where cells attach to single struts distant from each other, while large pores further hinder cell migration to adjacent struts (257,361). Small pores are known to facilitate cell

migration and accelerate tissue formation due to smaller span gaps between struts (257,261). The Z8-d7 was the design with the smallest pores, but also the one with the highest surface area (mainly due to numerous and more pronounced nodes). Given that all scaffolds demonstrated a wide range of strut distance distribution with relatively large pores (>600 µm), it can be inferred that the combination of high connectivity and high surface of nodes favoured cell-to-cell communication and cell differentiation over proliferation due to the accumulation of numerous cells over distinct locations (i.e., the nodes) inside the dense scaffolds. This suggests that the combination of scaffold porosity and topology (i.e., connectivity) drives differentiation independently to proliferation.

RT-qPCR analysis further highlighted that both connectivity and strut density had a positive effect on osteoblast differentiation and ECM maturation. Increasing connectivity or strut density led to reduced porosity and mean pore size with a simultaneous increase in surface area which favoured the expression of collagen type-I as suggested by previous *in vitro* studies (362).

This study employed pre-osteoblasts which are known to differentiate into mature osteo-cells *a priori*. Thus, any differences in cellular behaviour found here should be attributed solely to scaffold designs. In fact, all scaffold designs supported osteoblastic differentiation and ECM formation and mineralisation as evinced by the positively expressed osteogenic biomarkers and the positive staining with calcein, respectively. Measurements of ALP activity, osteopontin, and osteocalcin indicate that both strut density and connectivity positively influenced cell differentiation. However, the expression of these proteins is known to be asynchronous, thus the time of their peak expression is key for comparing the progress of cell differentiation between designs (363).

During the pre-osteoblastic cycle, ALP is expressed when accumulated inorganic phosphate groups (Pi) are generated. Pi groups with calcium ions further deposit amorphous calcium phosphate or hydroxyapatite crystals within the secreted ECM (364,365). This is considered an early phase marker of bone tissue formation, while at later phases ALP expression reaches a peak (plateau) value and finally decreases indicating the mineralisation of ECM (330,366,367). This pattern was observed only in the d7 scaffolds (**Figure 7.12**), while the d3 scaffolds exhibited a continuously increasing activity of the enzyme suggesting either a lag in cell differentiation or a state of continuous ECM mineralisation.

Osteopontin is a multifunctional protein which is secreted early by pre-osteoblasts to mediate cell adhesion and migration; however, its expression levels peak in more matured osteoblasts as it regulates collagen organisation and minerals deposition in ECM (368,369). While studies in mesenchymal stromal cells have shown that osteopontin expression increases as days of culture progress (370).

Osteocalcin, on the other hand, is principally secreted from mature osteoblasts as its main role is the maintenance of mineralised ECM as it binds to hydroxyapatite crystal (371,372). Prolonged culturing of MC3T3 pre-osteoblasts has shown that osteocalcin levels continuously increase over the days of culture (367). Higher expression in any of these two proteins due to higher strut density (d7) can again be attributed to the beneficial role of small pores (i.e., low strut distance) and high surface area (362). Still, connectivity appeared to have the most critical effect in the expression of both osteopontin and osteocalcin regardless of strut density.

Scaffolds Z4-d7 and Z8-d3 which exhibited similar porosity and pore sizes induced similar cell growth and expression levels of osteogenic biomarkers. This can be considered an expected outcome as the above morphological characteristics are known to be detrimental to cellular behaviour. However, the parameters used to control scaffold design were connectivity and strut density. The existing results strongly support that, in all cases, increasing connectivity over fixed strut density favoured osteoblastic differentiation. This validated the initial hypothesis that high connectivity would benefit ECM organisation towards quicker maturation and mineralisation due to the presence of multiple cavities in

the 3D structure as suggested by the literature (268,271). High connectivity nodes were more pronounced than the low connectivity nodes, and as a result, they provided more available surface area at the junction of the struts for cells to accumulate and form ECM. After two weeks, the Z8 scaffolds demonstrated distinguishable bridging of multiple corners around the nodes, compared to the Z4 scaffolds where ECM was mainly formed around the struts.

Out of all the designs, the dense Z8-d7 combining the highest tested values of strut density and connectivity demonstrated the most accelerated osteoblastic and ECM maturation. This scaffold was the only one with a plateau in the ALP expression and highly expressed osteopontin and osteocalcin at the end of culture (day 28). At the same time, it led to the largest ECM formation (shown through Picrosirius Red staining) and the most matured ECM (highest expression of collagen type-I). These findings suggest that the ultimate goal in stochastic scaffold design should focus on maximising the values of both strut density and connectivity while assuring that the structure maintains stiffness in the range found in natural trabecular bone.

7.3.2. Thin versus thick struts

In a more concise study, the effect of strut thickness was evaluated using a scaffold with intermediate connectivity ($Z=6$) and low strut density ($d=3$ struts/mm³). These resulted in scaffolds with relatively high pores of comparable size, albeit greatly different porosity and stiffness. This design approach was selected to better isolate the effect of strut thickness. As discussed previously, porosity and pore size have a great impact on cell proliferation and differentiation, thus by using large pores the effect of porosity would be negligible.

The most important finding of this experiment was that thin-strut scaffolds induced more expended ECM formation (seen qualitatively through three imaging techniques) which was also accompanied by higher expression of the ALP and osteocalcin at days 7 and 14, respectively (seen quantitatively using cell lysates). This can be attributed to the higher surface area to structure volume ratio of the thin-strut scaffolds compared to the thick-strut scaffolds. As an equal number of cells adhered to both scaffolds, thin struts facilitated the communication of cells which later led to larger unified matrix regions. These observations highlight the importance of considering the available surface area relative to the volume of the structure, rather than the absolute surface area that most studies – investigating different pore sizes and porosities – consider.

Increasing surface area to volume has previously been shown to lead to linearly higher cell adhesion of MC3T3 in porous collagen scaffolds with pore sizes ranging from 95 to 150 μm (373). However, no difference in cell attachment was found in the tested titanium scaffolds which employed larger pores (900 and 1100 μm). In a more relevant study, using titanium scaffolds and MC3T3 cells, Torres-Sanchez et al (258) showed that increasing surface area to volume led to higher ALP activity, albeit with no apparent difference in cell proliferation. However, in this study, they compared scaffolds with completely different topologies (periodic versus stochastic) and lattice types (sheet-based versus strut-based).

Other *in vitro* works investigating the effect of strut thickness primarily concerned electrospun scaffolds with nano-sized struts (374–376). Although these studies report that decreasing fibre diameter benefited cell differentiation and matrix maturation, the comparable size of the fibre to the cell size makes these studies irrelevant for comparison. In a recent *in vivo* study, Friedman et al (377) implanted stochastic strut-based titanium scaffolds built with 1300 or 650 μm struts in the femoral condyle of ovine models for a total of 12 weeks. However, no difference between the thick and thin struts was reported, albeit the difference in the elastic modulus.

Overall, this study might be one of the very few that investigate the effect of strut thickness in bone replacement scaffolds. Results suggest that considering smaller strut sizes while investigating other methods for providing a beneficial pore size (i.e., increasing connectivity and strut density) might be an optimal approach for maximising mechanical and biological performance.

8. Effect of post-processing on the mechanical and biological performance of CP-Ti lattices

8.1. Morphological changes due to surface treatment

Chemical-electrochemical treatment was applied to CP-Ti lattice structures initially printed with a strut thickness of 300 μm . Micro-CT scans were used to evaluate key morphological characteristics before and after the treatment with results summarised in **Table 8.1**. The post-processed specimens exhibited a strut thickness of ≈ 260 μm , meaning that the surface treatment resulted in a ≈ 40 μm reduction in strut diameter. The relatively low reduction in strut thickness was also accompanied by a small change in average roughness S_a (≈ 5 μm). However, a reduction of more than 50 μm was recorded by measuring the maximum peak-to-valley roughness S_z . This roughness measure captures the distance between extreme surface features, so a decrease suggests the removal of sharp peaks such as semi-sintered particles and other irregularities typically found in additively manufactured lattices.

Given these measurements, equivalent lattices were built with 260- μm struts to match the final strut thickness of the treated lattices. The equivalent lattices were used as-built for mechanical and biological tests as a direct comparison to the treated lattices. The equivalent lattices exhibited similar strut thickness (≈ 260 μm) and pore size (≈ 600 μm). In addition, since strut thickness did not exhibit drastic change, both S_a and S_z values of the equivalent as-built lattice were similar to the respective values of the initially printed lattice with the thick 300- μm struts. Moreover, the smoother surface in the treated lattices was translated to a higher surface area to structure volume ratio compared to the as-built equivalent lattice.

SEM imaging (**Figure 8.1**) was used to visualise the result of the treatment. Macroscopic images demonstrated that treated successfully removed the majority of semi-sintered around the struts and nodes. Smoothing was also noticeable at the downward-facing surface of struts, especially at low build angles, where typically numerous semi-sintered particles are fused forming dross. Qualitative observation through SEM suggested that surface finishing acted uniformly throughout the sample. This was also validated by the consistency in the measured strut thickness across all building angles.

The uniform reduction of thickness occurred along the length of the struts, meaning that surface treatment followed the profile of the original (as-built) struts. As a result, forms of roughness such as strut waviness and welding necks owing to consecutive melt pools remained after treatment. This form of roughness is comparable to the layer thickness (10s of μm) and the size of the melt pools, which may explain the average roughness $S_a \approx 10$ μm found in the treated lattices.

By examining the surfaces of structures at high magnification (**Figure 8.1**), drastically different surface topographies were able to be distinguished between the treated and as-built lattices. Specifically, both as-built lattices exhibited relatively smooth topographies with irregularities being the micron-scale semi-sintered particles and nano-scale texture lines formed due to the overlap of build-layers and melt-pools (114). On the contrary, the treated surfaces exhibited a rough topography consisting of dimples. Similar morphologies have been seen when CP-Ti discs were chemically etched using sulfuric acid

(H₂SO₄) at room temperature (similarly to the temperature used here), but also when subjected to sandblasting (378).

Last, it should be noted that for consistency all structures were built using the same laser energy (200W). In addition, strut thickness in the treated and the two as-built lattices did not exhibit large differences, similar energy densities were used during the additive manufacturing, resulting in similar internal porosity ($\approx 0.01\%$) in all samples that were micro-CT scanned.

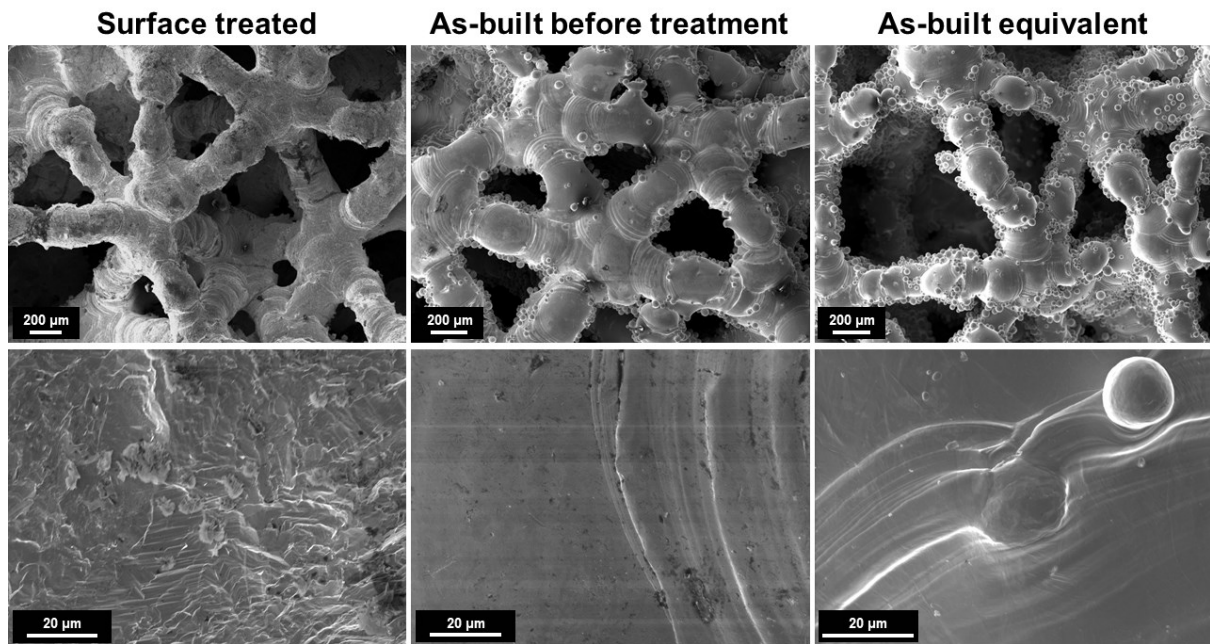


Figure 8.1. The effect of surface treatment on structure morphology. Images were taken with SEM at 5kV and 1nA. The specimens were arranged in columns: the first row shows the structures at a macroscopic level, while the second row presents magnified views of distinct regions on the struts.

Table 8.1. Morphological characteristics of as-built and surface treated structures <i>Measurements were taken using micro-CT scans unless otherwise stated.</i>			
Properties and feature sizes	Etched	As-built Before Etching	As-built Equivalent
Porosity [%]	71	67	71
Strut distance [μm] *	560 ± 90	Not measured	600 ± 90
Strut length [μm] *	910 ± 150	910 ± 150	910 ± 150
Surface area to volume ratio [mm^{-1}]	13.66	Not measured	12.18
Effective strut thickness [μm] (n=20)	263.2 ± 17.4	302.0 ± 13.4	260.2 ± 24.3
Average surface roughness S_a [μm] **	10.3 ± 2.9	14.9 ± 3.5	15.5 ± 2.8
Max peak-to-valley roughness S_z [μm] **	93.8 ± 25.4	152.9 ± 30.3	169.4 ± 53.5

* Values taken from CAD model and rounded to the nearest 10.

** Values were taken by measuring 20 randomly picked struts built at different angles.

8.2. The effect of surface treatment on mechanical performance

Treated and as-built lattices were tested under quasi-static compression and then cyclic compression-compression. A summary of the quasi-static properties is provided in **Table 8.2**.

Table 8.2. Static mechanical properties of as-built and surface treated structures <i>Measurements were taken using n=5 physical specimens per condition unless otherwise stated.</i>			
Properties and feature sizes	Treated	As-built Before Etching	As-built Equivalent
Nominal strut thickness [μm]	260	300	260
Nominal t/L ratio *	0.29	0.33	0.29
Relative density [%]	27.6 ± 0.1	33.8 ± 0.9	26.8 ± 0.5
Compressive strength [MPa]	57.2 ± 1.5	66.7 ± 4.3	40.5 ± 0.7
Elastic modulus [MPa]	6772.3 ± 211.4	7792.9 ± 252.6	4721.4 ± 109.1

* Values were taken from micro-CT data and CAD model.

Although the particular stochastic lattice design was not tested before, the porosity of the as-built parts made with 260- μm and 300- μm struts perfectly match the predictive relative density provided for $Z=8$ in **Figure 5.1** for t/L of 0.29 and 0.33, respectively.

Treated lattices exhibited lower relative density compared to the as-built condition prior to treatment. This led to lower compressive strength and lower elastic modulus. It is important to highlight that the as-built condition with the 300- μm exhibited a stiffness that is typically found in cortical bone. However, after treatment the stiffness of the structure reached the limit between cortical and cancellous bone ($\approx 7\text{GPa}$). The high stiffness was a compromise in order to achieve a mean strut distance – which was used as an approximation of pore size – near 600 μm that is considered beneficial for bone in-growth.

Compared to the as-built equivalent lattices, the treated structures exhibited similar relative density but with critically higher mechanical properties. In particular, the surface treated structures exhibited 35% higher strength and 14% higher stiffness, although made with equal strut thickness.

In cyclic compression (**Figure 8.2**), both the normalised and absolute fatigue strength of the treated lattices was superior to that of the as-built equivalent lattices. Interestingly though, the treated samples exhibited similar fatigue performance to the initial as-built lattices although exhibiting lower relative density and quasi-static strength. While both as-built lattices experienced catastrophic failure at the 30% test level, the surface treated lattices withstood over 2 million cycles and tests were terminated without failure. By comparing the slopes of S-N curves it can be assumed that the treated lattices can outperform the as-built lattices with the 300- μm struts in the high-cycle fatigue life ($>10^6$ cycles).

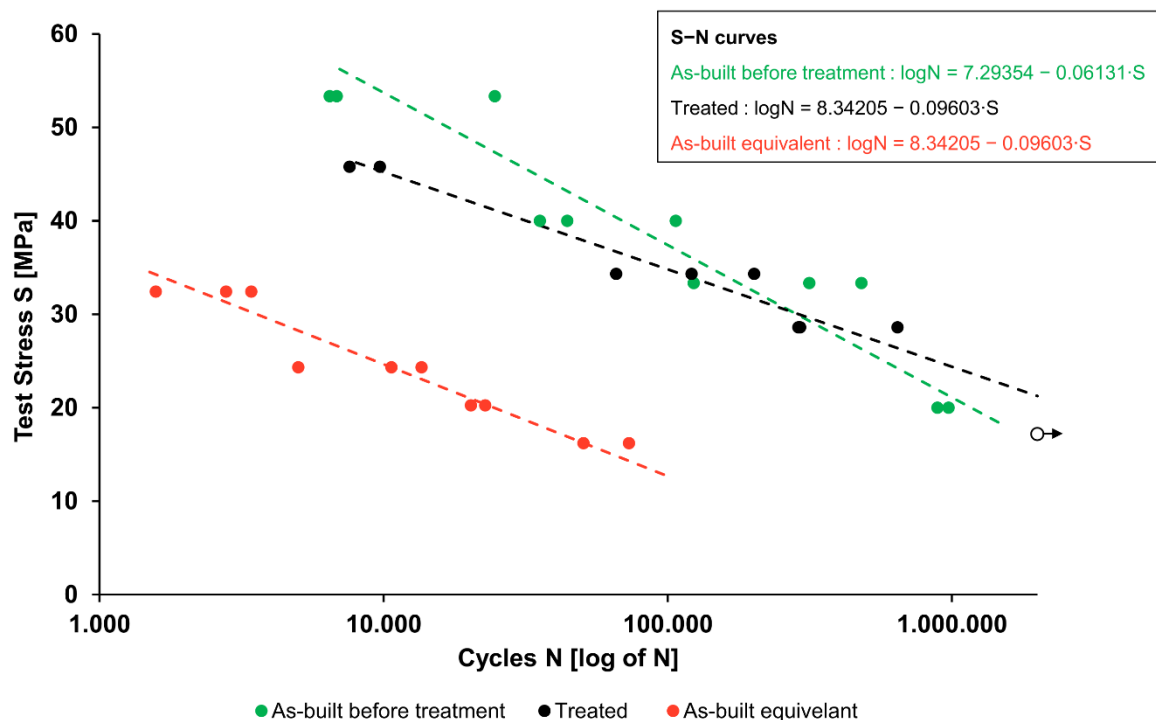


Figure 8.2. The effect of surface treatment on cyclic compression. The S-N curve for the treated specimens was calculated without the data points at 30% test level since tests were terminated before catastrophic failure.

8.3. The effect of surface treatment on biological performance

Scaffolds that were subjected to surface treatment and untreated scaffolds printed with equivalent strut thickness were used for cell culture. The choice of including only these two design variables was based on the equal strut distance (pore size) and surface area to volume ratio that these two exhibited (**Table 8.1**).

Two measurements were used to assess the effect of surface treatment on hMSC function: cell viability (measured through cell metabolism) and ALP activity (**Figure 8.3**).

No statistically significant difference in cell metabolism was observed between the treated and untreated scaffolds ($p > 0.05$), suggesting comparable cell seeding efficiency and metabolic activity between both conditions. However, statistical analysis revealed that the culture day had a significant effect on cell metabolism ($p < 0.001$), indicating an increase in cell activity over time. Both scaffold conditions exhibited a similar 150% increase in metabolic activity by day 6, consistent with expected cell growth.

In both scaffold types, ALP activity exhibited a trend of increasing and then decreasing ALP with a local maximum around 13 days. Statistical analysis further indicated that the ALP activity (normalised to total protein) was significantly higher in the treated compared to the untreated scaffolds ($p < 0.001$).

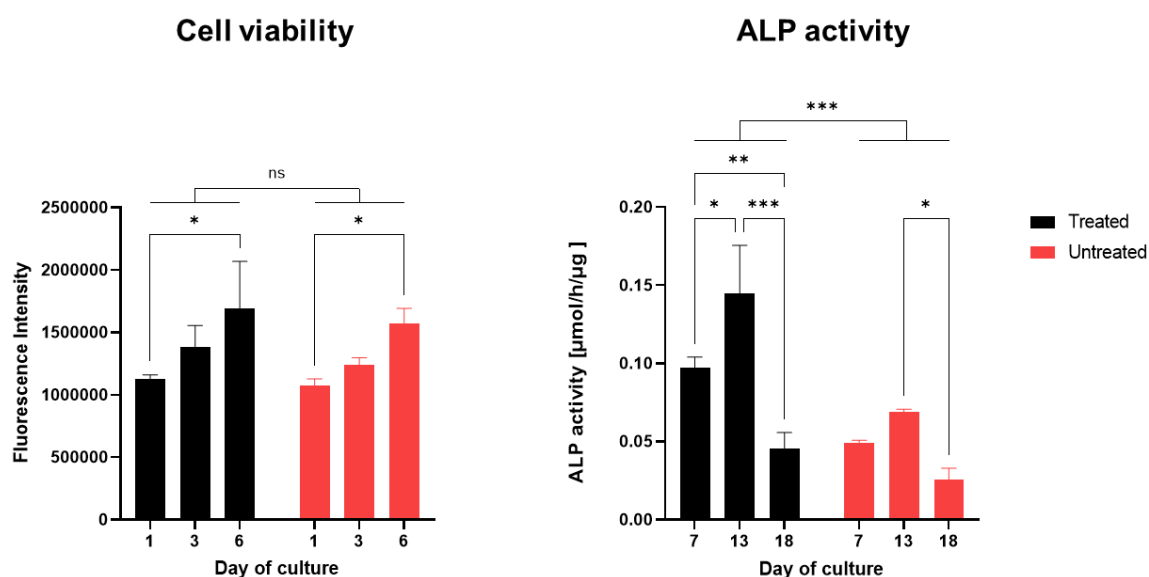


Figure 8.3. The effect of surface treatment on hMSC viability and differentiation. A sample size of $n=3$ was used in both assays.

Both scaffold types were successfully stained with calcein – known to bind to calcium crystals – indicating the initiation of the mineralisation process (**Figure 8.4a**). Measuring the calcein-stained area on scaffold surfaces provided a direct comparison of mineral deposition within the two scaffold types. This analysis showed that the surface-treated scaffolds induced significantly higher ($p < 0.05$) mineral deposition compared to the untreated scaffolds (**Figure 8.4b**).

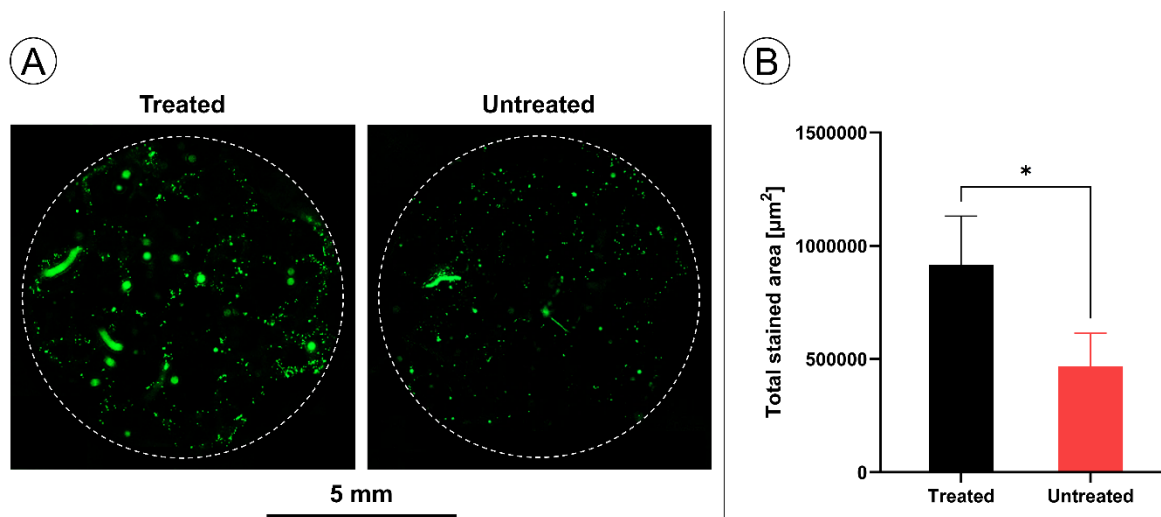


Figure 8.4. The effect of surface treatment on calcium crystal deposition. (A) Representative fluorescent images of calcein labelling. Green areas indicate calcium-rich regions on the surface of surface-treated and as-built (untreated) titanium scaffolds. White dashed circles indicate the scaffolds' boundaries. (B) Quantification and comparison of the calcein-stained area on scaffolds. A sample size of $n=3$ unique specimens was used.

8.4. Results interpretation

The chemical-electrochemical surface treatment (Hirtisation®) was previously tested in Ti-6Al-4V lattice structures (227), reducing the average strut surface roughness (S_a) from 12 μm to 6 μm and reducing strut thickness up to 100 μm . In the current work, CP-Ti lattices were treated using the same process conditions and strut thickness reduction was only 40 μm . Although surface irregularities were mostly removed, as suggested by the S_z measurements. The macroscopic profile of the strut was retained, thus roughness defects such as weld-necks and waviness remained.

In terms of mechanical performance, the treated lattices appeared superior to the equivalent as-built counterparts under both quasi-static and cyclic loading. This can be attributed to the homogenous thickness along strut length, leading to higher load-bearing capacity (226), as well as the smoother macroscopic surface and the absence of surface defects, i.e., semi-sintered particles, known to greatly comprise fatigue strength (224). Notably, the treated lattices exhibited similar normalised and absolute fatigue strength to the initial lattices with 300- μm struts. And in turn, this can be attributed to the relative low strut thickness reduction seen in the treated lattices, which helped them maintain the structural benefit of thick struts while incorporating reduced structural defects.

The effect of surface treatment on the biological performance of titanium scaffolds was investigated through the *in vitro* culture of hMSCs on surface-treated and as-built scaffolds with equal strut thickness. While the surface treatment did not induce significant differences on cell adhesion or proliferation (estimated through cell metabolism), it significantly enhanced osteogenic differentiation, as evidenced by increased alkaline phosphatase (ALP) activity and calcium deposition.

In both scaffold types, ALP activity followed a typical temporal profile, peaking at day 13 and declining by day 17. This pattern aligns with the established progression of osteogenic differentiation, as previously discussed. The observed ALP profile is critical for two reasons: first, it confirms the successful

commitment of hMSCs to the osteogenic lineage, and second, it reflects the transition of cells toward osteoblastic maturation and matrix mineralisation.

Surface-treated scaffolds exhibited consistently higher ALP activity across all time points, suggesting that surface treatment enhances the osteoconduction of pure titanium. Furthermore, the significantly greater calcein-stained area on treated scaffolds indicates that the elevated enzymatic activity is directly linked to more robust mineral deposition. These findings imply that the chemical-electrochemical surface treatment creates a surface topography more conducive to cell differentiation and the deposition of hydroxyapatite-like minerals, a critical step in the bone formation process.

9. Discussion

9.1. Selecting design parameters for bone-matching properties

The first part of the thesis demonstrated that nodal connectivity Z , strut density d and strut thickness t can independently define a stochastic structure during the design phase and predict the relative density, stiffness, and ultimate strength of the structure. This unified methodology was demonstrated over a wide range of porosity (60% to 90%) and stiffness (0.1 to 8 GPa), using 4 connectivity levels (4, 8, 12, 14 struts/node), 3 strut densities (3, 5, 7 struts/mm³) and two strut thicknesses (230 and 300 μ m).

Structures with high connectivity ($Z \geq 8$) exhibited more stretch-dominated characteristics than low connectivity ($Z=4$) and had higher ultimate strength and stiffness for a given relative density. Connectivity was shown to independently affect relative density and was a key parameter in fatigue loading. Increasing connectivity increased fatigue strength for a fixed relative density owing to the associated higher ultimate strength. These findings are important because they show how a stochastic lattice can be tuned to act either as a bend-dominated foam with a constant post-yield plateau, or a stretch-dominated lattice with high static and fatigue load-bearing capabilities. The advantage of the stochastic structure is that the single integrated model, presented in this study, can define the structure to achieve a broad range of design requirements, even as a gradient of properties within the same component. To achieve the same with periodic lattices would require different unit cells, with individual regression models for each unit cell used.

Numerous published studies can be found in literature where AM periodic lattices have been tested in quasi-static compression. **Figure 9.1a** illustrates the wide range of stiffness values (100 MPa to 8 GPa) that can be manufactured from a stochastic structure that is governed by the unified relationships presented in this study – this is a wider range of values than has previously been reported in a single study. Yet, most studies available employ periodic structures made from Ti-6Al-4V alloy, while in this work the more ductile CP-Ti was used. To allow comparison, the relationship between ultimate strength relative to the parent material and stiffness relative to the parent material is illustrated in **Figure 9.1b**. This comparison shows that the stochastic structures presented in the current work exhibit equal or higher strength/modulus relationship compared to most previously assessed periodic structures (113,160,175,379,380).

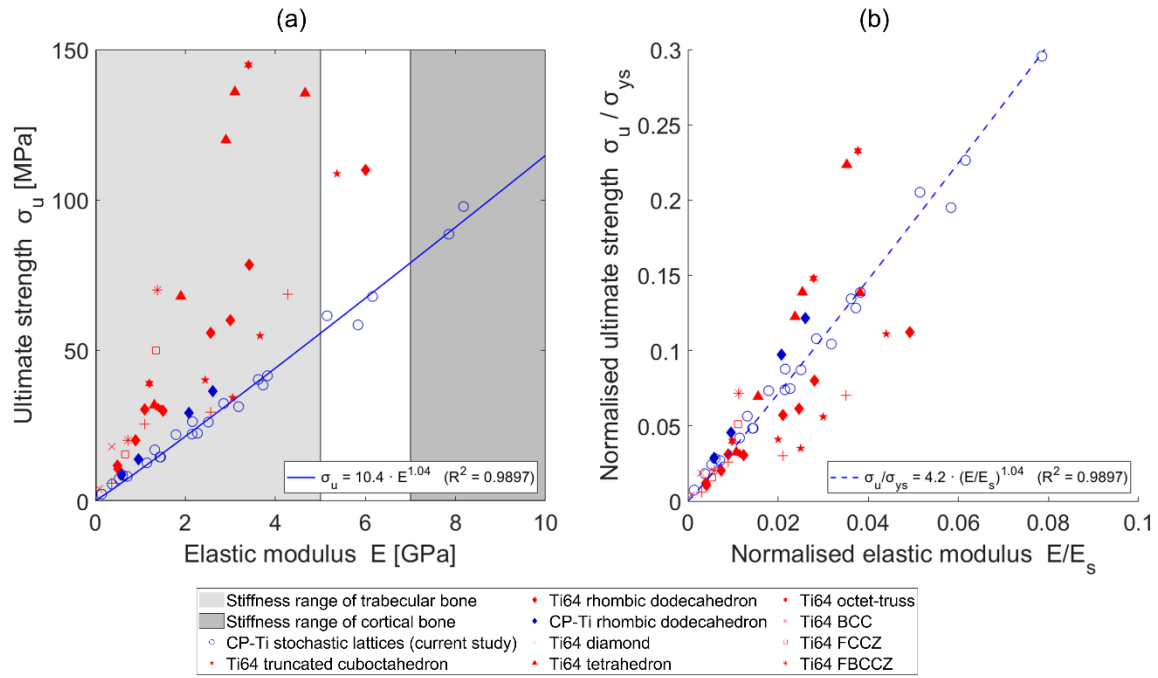


Figure 9.1. Comparison of strength-to-modulus ratio in stochastic and periodic lattice structures. Structures in this work were made using CP-Ti, while literature data consist of structures from CP-Ti or Ti-6Al-4V. Measured mechanical properties are presented in (a) absolute values and (b) normalised to solid material properties. A power law regression model was used to correlate stiffness to strength using all data points from this study. The mechanical properties of lattice and solid specimens were taken from (113,160,175,379,380). Figure reproduced under CC BY license from (109).

Regarding fatigue testing, the normalised fatigue strength (σ_f/σ_u) of 17%-22% for the tested structures compared well to previous works. Normalised fatigue strength of 15% to 25% has been reported for Ti-6Al-4V lattices with the diamond unit cell and relative densities of 17% to 40% (158). Normalised fatigue strength of <20% has been reported for Ti-6Al-4V lattices with the dodecahedron unit cell and relative densities from 16% to 32% (172). In AM stochastic lattices built with Ti-6Al-4V, Ta and CP-Ti, normalised fatigue strength was found to range from 15% to 30% (114). In other cases, however, much higher normalised fatigue strength, between 30% to 55%, have been reported for various lattices built with both CP-Ti (103,175) and Ti-6Al-4V (381), but these estimates may not be realistic given the normalised fatigue strength of solid CP-Ti and Ti64 is ~66% (114) and ~40% (172), respectively. Overall, it seems that the tested structures behaved comparable to what other works have shown. However, just like quasi-static, the fatigue strength cannot be examined as an independent property. The high absolute fatigue strength of a scaffold should be accompanied by a reasonable porosity and appropriate stiffness.

This study demonstrated that connectivity can increase fatigue strength up to 60% over constant relative density. Although it did not show that fatigue strength can be maximised above previously reported limits, there is room for improvement. High-cycle fatigue life is highly influenced by structural and morphological defects associated with AM. Semi-sintered particles and texture lines can act as stress concentration and crack initiation sites, especially near the nodes (114).

9.2. The interplay between lattice dimensions and topology

The second study presented here verified that lattices' mechanical properties are constant for aspect ratio between 1 and 2 as suggested by international testing standards, however, they can vastly differ outside this region. The different trends of convergence in stiffness, strength and energy absorption for different lattice types support the notion that extended mechanical characterisation should be done prior to designing parts whose geometry falls outside this aspect ratio.

Of particular interest are fully porous orthopaedic implants designed to locally match the properties of natural bone, and implants can potentially have high aspect ratios (e.g., femoral stems) or low aspect ratios (e.g., knee replacement components) (382,383). In addition, the lattice sections of implants may consist of multiple layers and unit cells (in the case of large implants like femoral stems), but in most cases, they consist of few layers. Representative examples of the latter are lattice-based pedicle screws (384) (high aspect ratio structure with diameter <6mm), spinal fusion cages, acetabular cups, and tibial tray components (low aspect ratio structures with a total thickness of 3-4 mm) (383,385,386). Another design approach, with potential application to orthopaedic implants, would be functionally graded structures where different lattice topologies of varying aspect ratios can be stacked together to induce locally varied deformation behaviours – alternatively to previous concepts where only unit cell size or type was varied (387–389).

The results presented here highlight that the selection between stretch-dominated or bend-dominated lattice structures should consider the aspect ratio of the application. Stretch-dominated lattices may better suit low-aspect ratio applications as their stiffness remained constant for decreasing aspect ratio. In contrast, bend-dominated lattices may better suit high-aspect ratio applications as their stiffness remains constant for increasing aspect ratio. It is however noted that, based on the two topologies tested, bend-dominated lattices seem to be more prone to buckling which can potentially limit their compatibility for extreme aspect ratios (e.g., very elongated components like pedicle screws).

Another important remark regards the coupled effect of size effects and aspect ratios. Early studies have shown that foams and trabecular bone specimens can be very sensitive to decreasing size, resulting in a drastic reduction of mechanical performance. The results of this study, however, suggest that additively manufactured lattice structures can retain their mechanical properties constant even when designed with 3-5 layers of unit cells regardless of aspect ratio. While the stiffening mechanisms for stretch and bend-dominated lattices also remain unaffected by size effects.

9.3. Selecting design parameters for enhanced osteogenesis

The vast majority of cementless implants that are currently used in clinical practice employ stochastic lattice structures. This preference can be attributed to their bone-like architecture which benefits cellular function and tissue formation and facilitates the representation of arbitrary component shapes while maintaining high isotropy.

In vivo animal trials (39,390) suggest that bone in-growth and the quality of regenerated tissue (lamellar versus woven bone) highly improve when scaffold stiffness matches the stiffness of natural bone. This is attributed to the positive bone remodelling of host bone due to the generation of physiological strains upon implantation. Critical-sized implants, however, are subject to gradient loads thus they should combine regions of high and low porosities. Lattice designs with locally graded porosity to match the stiffness of bone (48,391) and induce high strains to the host bone (392) have been previously proposed

to mechanically stimulate osseointegration. In most cases, periodic lattices with varied strut thickness or unit cell type/size were used. However, these approaches require a variety of known laser parameters to fabricate several strut thicknesses or introduce issues of unit cells mismatch (109,393). Varying strut thickness remains the gold standard for generating graded structures, however rigorous mechanical testing of each set of laser parameters is required to accurately control strut and structure properties (199,394).

The proposed design model offers the flexibility of designing bone-matching lattices by varying three design parameters: connectivity, strut density and strut thickness. In particular, structures with stiffness similar to trabecular and cortical bones could be developed under different aspect ratios and lattice sizes. In practice, this is a major advantage because it requires manufacturing validation for only a few strut thicknesses to generate parts with the range of properties needed in the orthopaedic sector.

Nodal connectivity in natural bone ranges between 3 and 5 (276), thus the majority of stochastic lattices used in commercial implants or academic research are designed with low connectivity (26,39,282). Increasing connectivity from the 3-4 struts/node typically seen to the proposed high 8-14 struts/node transitions the lattice behaviour from a bend-dominated to a stretch-dominated state. Independent Gibson-Ashby models were developed for each case (low and high Z) and were shown to accurately predict stiffness and strength for lattices used in different studies throughout this thesis. Previous experimental data from this and other works (109,160,175) suggest that increasing connectivity can significantly improve the static and fatigue strength owing to the more tightly interconnected struts. Moreover, the third part of this thesis demonstrated that high connectivity positively influences cellular behaviour as it provides high surface area and multiple concavities around the nodes for cells to aggregate and quickly form mature bone tissue.

Strut density was selected to lay in the range of trabeculae density (typically up to 10 trabeculae/mm³) found in natural bone (395). This successfully led to structures with sufficient porosity for the majority of Z , d , and t combinations used in this study. Still, a relatively high strut density, close to 7 struts/mm³, appeared necessary to provide pore sizes close to the optimal 600 μm .

High load-bearing lattices are typically designed with decreased pore size by increasing strut density. Extreme strut densities accompanied by low connectivity will eventually lead to very small pore sizes that will hinder cell migration and vascularisation *in vivo* (356,396,397) while the design of large-scale fully porous implants requires generally considerably higher computational times. As a proof-of-concept, Burge et al (383) utilised the same lattice model to develop lattice-based tibial trays with patient-specific bone-matching stiffness based on CT scans. The computational time for the design of the lattice increased exponentially with increasing strut density (**Figure 9.2**). However, the use of high connectivity ($Z=8$) appeared to decrease computational time compared to low connectivity ($Z=4$), especially for high strut densities.

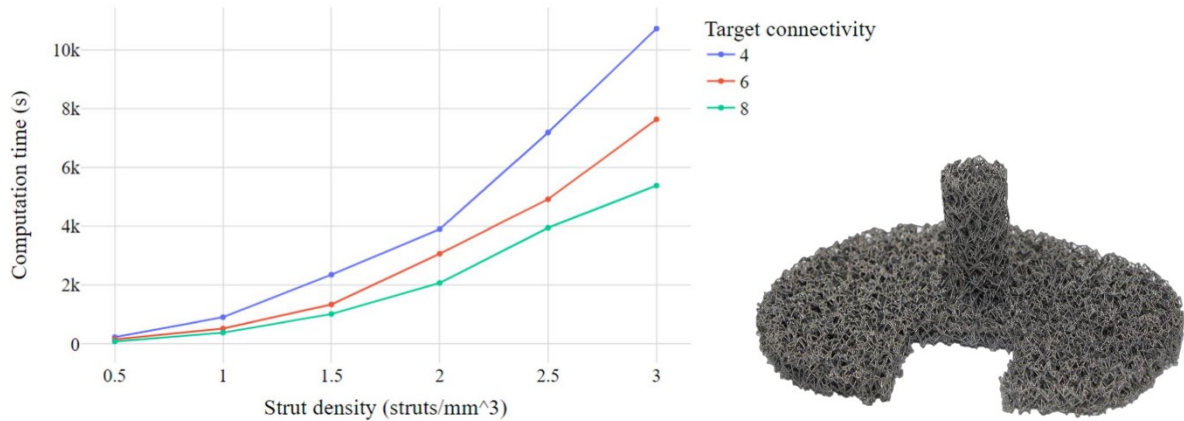


Figure 9.2. Computational times for the development of large-scale porous knee implants using the developed stochastic model. Figure reproduced from (383) with permission from Springer Nature (License No. 5975210362155).

This *in vitro* study was also one of the very few that have investigated the effect of strut thickness. Results highlighted the importance of considering the surface area relative to the structure volume which increased for decreasing strut thickness. Higher surface area to structure volume ratio facilitated cell communication and greatly accelerated tissue maturation within stochastic lattices. However, very thin struts are challenging to fabricate and greatly compromise the fatigue strength. The latter was shown from both fatigue analyses presented in this thesis by testing Z4-d5 lattices with 230- μm and 300- μm struts and Z8-d8 lattices with 260- μm and 300- μm struts. 170 μm was the smallest possible strut thickness that could be reliably produced using the existing powder bed fusion machine. Typically thought struts in titanium scaffolds are made $>200\ \mu\text{m}$ to ensure printability and reproducibility for different machines and higher mechanical strength.

Collectively, these data suggest that stochastic lattice structures should be built with a relatively low strut thickness within the range of trabeculae thickness, approximately 150-300 μm (398,399), to balance high strength and high surface area to structure volume.

Throughout this thesis, connectivity has been proven a key parameter for lattice mechanical behaviour and biological response. Higher connectivity ($Z \geq 8$) benefits osteogenesis and significantly increases the lattice's fatigue strength which is a crucial parameter for developing load-bearing implants for joint replacement. Hence, using a strut thickness between 200 and 300 μm , designing stiffness-matched lattices with increased connectivity and adjusted strut density constitutes a novel and efficient method for combining high mechanical endurance while allowing room for deep bone in-growth and vascularisation.

9.4. Post-processing for orthopaedic implants

The last part of the thesis aimed at developing the optimal lattice according to the previously discussed remarks, while combining a surface finish to benefit fatigue strength and osteoconduction. A novel hybrid post-process method was used to overcome known limitations of conventional chemical etching and electropolishing.

Lattice structures were designed with high connectivity ($Z=8$) and strut density ($d=8$ struts/mm³) to combine high strength with a biomimetic porosity (>70%). The surface treatment led to a confined reduction of strut thickness (40 μ m), allowing the preservation of structural integrity while removing the majority of protruding semi-sintered particles which are known to impair fatigue strength. In line with literature, surface post-processing led to considerable increase in the fatigue strength of treated lattices compared to as-built lattices of similar strut thickness. The final lattice employed a desired pore size of 600 μ m, which is widely believed to optimise osteogenesis (396,400), and a stiffness within the range of trabecular bone (7GPa) (46).

From a biological perspective, smoothening of strut morphology has been shown to increase surface wettability and hydrophilicity (303) which is critical for cell adhesion. Previous works employing pre-osteoblast cell lines (MG63 and MC3T3) has shown no or minimal differences on cell proliferation and differentiation between as-built and chemically etched scaffolds (303,401). In this work, human mesenchymal stromal cells (hMSC) were seeded on surface treated and untreated scaffolds with the surface treatment having no effect on cell adhesion or proliferation, but an apparent effect on cell differentiation and mineralisation. The use of human primary cells aimed on making this *in vitro* experiment more clinically relevant. In fact, these results are perfectly in line with O'Keefe et al (223) who reported that chemical etching had minimal effect on hMSCs proliferation, but induced higher matrix mineralisation when scaffolds were subjected to chemical etching.

Nevertheless, it is important to underline that *in vitro* data are particularly helpful to understand the effect of surface treatment on the cellular level. Extended *in vivo* experiments and clinical trials are more suitable to draw exclusive conclusions regarding the effect of surface treatment on osseointegration. While such studies are missing from existing literature, using the observations of this thesis alongside data from previous studies can lead to some useful remarks regarding the role of surface roughness in lattice-based implants.

In the case of on-growth surfaces, roughness is determinative for the implant's initial fixation due to higher friction with bone (402,403) and later biological fixation through osseointegration (404). However, when using lattice structures, implant long-term stability depends on bone in-growth and the effect of strut roughness appears to have either neutral or negative impact on osteoblast activity. Early *in vitro* models have shown that surface roughness benefits cell adhesion, proliferation, and differentiation when it is comparable to cell size (≈ 10 μ m) (284–288). In fact, this micro-scale roughness that cells can sense was seen in the topography of treated samples which exhibited micro-dimples. Nevertheless, strut roughness in all scaffolds tested as-built here was mainly above this limit, with the semi-sintered particles acting as surface discontinuities which led cells to cluster and secrete ECM around them, thus disrupting their communication with adjacent cells.

Overall, holistic examination of the currently available data from this work and literature advocate towards the removal of semi-sintered particles from additively manufactured lattices. First, surface treatment undoubtedly improves mechanical performance under cyclic loading. Second, it can benefit cell differentiation owing to a micro-rough topography at the strut surface that cells can sense and can lead to high protein adsorption. Last, owing to the absence of protruding semi-sintered particles, it can minimise the chances of bone resorption that can happen *in vivo* due to floating metallic debris from wear (405).

9.5. Limitations and direction for future works

Overall, this thesis constituted an extensive collection of mechanical and biological analyses which led to valuable insights on the designing, evaluating, and processing of lattices structures for improving the technology of cementless implants. Nevertheless, all studies presented here exhibit some limitations which can be addressed in future works.

9.5.1. Mechanical testing

On controlling the mechanical behaviour of stochastic lattice structures

In **Study I**, a unified model combining connectivity, strut density and strut thickness was developed to predict the relative density of stochastic lattice structures, which was then used in the Gibson-Ashby model for predicting strength and stiffness.

Firstly, the anisotropy of structures was not quantitatively represented or used in the model. In previous studies where stochastic lattices were developed using the same additive manufacturing setup (89,122), low-angled struts ($<30^\circ$) were trimmed making the structures more anisotropic and stiffer along the vertical $<0,0,1>$ direction of uniaxial compression. Lattices used here possessed struts in all directions and the distribution of struts with respect to angle was similar for all designs: approximately 50%, 35%, and 15% of total struts corresponded to 0° - 30° , 30° - 60° and 60° - 90° angle segments with respect to the x-y plane, respectively.

Although structures may have exhibited similar isotropy, small differences in the degree of anisotropy may affect the accuracy of property predictions. The interplay of relative density and anisotropy on predicting the mechanical properties of cellular materials has always been a topic with high scientific interest. In trabecular bone, early studies have demonstrated that incorporating porosity, trabecular spacing and anisotropy ratio has led to multivariable linear regression models that predict the mechanical properties with high accuracy ($R^2 > 90\%$) (406). Thus, a parameter that quantifies anisotropy could be integrated in the proposed regression model to make it more realistic. On the same time, this complex interconnection of all design and structural parameters clearly demonstrates the need for developing lattice structures with fixed anisotropy and as close to isotropy as possible for accurate prediction of mechanical properties.

Secondly, only uniaxial compression tests were carried out in this work, additional tests involving multi-axial loading and different loading types (tensile, bending) would be beneficial for better structure designing for industrial or clinical applications where structures experience multiple loading types.

Lastly, higher order or non-linear expressions could have been more realistic for linking the design parameters to structure properties. Thus, the developed linear relationship may not be representative outside the range of values included in this study. However, the strong correlation found for the linear model ($R^2 > 90\%$) indicates that this approach was acceptable for the considered range of variables.

On the effect of aspect ratio for different topologies and coupon sizes

To investigate the effect of aspect ratio, four widely used lattice topologies were tested with different relative densities (ranging from 6% to 30%), specimen geometry (cylindrical versus prismatic) and micro-architectures (stochastic versus periodic). Each topology demonstrated either a bend or stretch-dominated micro-deformation mode, which is known to be independent of relative density and strut slenderness (strut thickness-to-length ratio) for fixed base material (109,344,407). Yet, different base materials have been shown to induce different failure modes for the same lattice designs due to their intrinsic ductile or brittle nature (114). For example, the bend-dominated BCC has been shown to

fracture through shear banding when made of the brittle Ti-6Al-4V (408), counter to what was observed for the pure titanium BCC lattices here. Nevertheless, the stiffening of our BCC lattices with decreasing aspect ratio agrees with previous computational data for bend-dominated lattices (146,149) using the Ti-6Al-4V. Although this primarily verifies that the stiffening at low aspect ratios applies to bend-dominated structures, validation and global extension to other material types are left for future work.

The stiffening mechanism of the BCC lattices was found to become less apparent with decreasing relative density and slenderness ratio. This informs that relative density can also influence the effect of aspect ratio for a given topology. A future investigation for a wider range of relative densities will further enrich our knowledge regarding the effect of aspect ratio. In this case, however, a more rigorous analysis of the interplay of structural defects on the mechanical response may be necessary.

Small-sized specimens were used to further explore the coupled effect of aspect ratio and size. Based on experimental observations, it was shown that some lattice topologies are more prone to size effects (bend-dominated BCC) while others do not seem to encounter size effects (stretched-dominate octet-truss). The results led to interesting insights to better understand the scalability of lattice structures, however more extensive research regarding the strain distribution within small-sized structures is still needed. DIC imaging was not easy to be implemented in such small sized objects, thus a computational model (409) would be more appropriate for providing valuable information regarding the loading state of each strut within the structures and unfold possible edge effects that lead to currently ambiguous stiffening mechanisms.

DIC was used to capture strain accumulation during the compression testing. 2D DIC was unable to capture out-of-plane deformation of external surfaces for the varying geometries. However, DIC was only used as a means of assessing the dominant deformation patterns of the different structures during compression testing. Due to the nature of compression testing, some strut features were not tracked in the later stages of plastic deformation – after cell fracture or densification. Nevertheless, the patterns necessary to understand the deformation were present in the early stages of plastic deformation (plateau region). Volumetric characterisation techniques, such as Digital Volume Correlation (410), can surpass current limitations and enable the calculation of deformation in multiple directions and properties such as Poisson ratio, shear and bulk modulus.

9.5.2. Biological testing

The image-based analysis presented has several limitations. Firstly, observations emphasized on the distribution of cells and ECM mainly on the top layers and the periphery of scaffolds. Cutting the specimens and observing the extent of cell penetration and tissue formation towards the middle of the scaffolds would provide valuable insights for the development of large-scale porous implants. However, this would require fixation in epoxy resin to avoid damage of the cellular structure and the scaffold material due to high-speed cutting. Secondly, monitoring cell proliferation and ECM expansion along struts and around nodes at higher magnification would better illustrate how topology and strut morphology guide tissue formation. Such study, however, would require numerous timepoints.

The initial cell seeding density is critical in 3D cell cultures, where cells appear to grow and proliferate slower than in coated well plates (411). Literature data (354,355) suggest that noticeable ECM formation and mineralisation in 3D cell cultures requires seeding densities $>1 \times 10^6$ cells/cm³. This standard was met in the cell cultures employing the MC3T3 which are known to proliferate and mature quickly. A relatively low cell seeding density was employed in the cell culture using the hMSC ($\approx 5 \times 10^5$ cells/cm³) which might have compromised cell proliferation, matrix formation and differentiation. Primary cells are known to proliferate slowly. Growth factors dosages and cocktails were used for the 3D culture known to promote osteogenesis, as well as enhance cell proliferation (412,413). Higher cell numbers could have

achieved if FGF and IGF were added from the proliferative phase in flasks. While a preliminary study focusing on optimising the proliferative and osteogenic media would be beneficial.

9.5.3. Considerations for future

Post-processing treatments applied here (i.e., NaOH etching and chemical-electrochemical treatment) benefited major cell functions such as adhesion, proliferation, and differentiation. The application of biomimetic coatings would further enhance the bioactivity of scaffolds. Techniques such as plasma spraying, electrochemical deposition and sol-gel have previously been used to coat solid titanium implants with hydroxyapatite or calcium phosphate compounds (414) and could potentially be applied to lattice structures too. Peptides for enhancing cell adhesion and guiding differentiation would also be an interesting alternative.

The *in vitro* experiments were cell-only models which emphasized on the influence of lattice topological parameters on cellular behaviour. These experiments aimed to be as detailed as possible by studying novel designs at reasonable time spans. A variety of different techniques collectively supported the raised arguments through complementary optical and quantitative means.

Findings of this work highlighted the impact of lattice topology on cell proliferation, matrix deposition and maturation. High connectivity was found to promote cell differentiation due to a combination of high surface area and numerous concavities around the nodes. Numerous corners facilitated matrix deposition and expansion especially when combined with small pores and thin struts that enhance cell interactions. However, these observations were made at a macroscopic level, limiting insights into individual cell behaviour. The image analysis used did not capture cell mobility, making it unclear whether cells migrated within the structure or preferred bridging high-connectivity nodes over wrapping around thin struts. Future work should utilise single-layer scaffolds to better assess how cells interact with scaffold's topological features, providing essential insights into *in vivo* cellular behaviour.

However, cell-only models are limited in that they do not consider cell migration from native tissue, the influence of physiological loading, or vascularisation. A next step could be to consider how cells migrate from bone tissue into lattices and the effect of physiological loading using an *ex vivo* model (235). Hypoxic culturing conditions should also be implemented in future lab-based works to better simulate the environment of natural bone – also known to regulate osteo-angiogenesis (415,416). Ultimately, the interplay between bone formation and vascularisation needs to be examined with an *in vivo* model.

More *in vivo* data are needed to outline how scaffold morphology and surface properties affect the bone-implant interface and how the quality of tissue formed at this interface is changing over long periods of healing (417,418). Additionally, while the use of cell-seeded scaffolds has previously shown promising results for *in vivo* bone regeneration in biodegradable scaffolds (419,420), there has been a lack of recent investigation of pre-seeded titanium implants.

Finally, the results suggest that all scaffold designs can support bone-like tissue formation, albeit with different *in vitro* maturation time. Yet, the porous structure and properties of trabecular bone varies depending on the anatomical site, age, sex, activity level, and health condition (48). Consequently, future research should also focus on the production of anatomical site or patient-specific implants by investigating how topologically different stochastic scaffolds perform depending on the nature of the host bone.

10. Conclusions

This thesis aimed to investigate the impact of additively manufactured lattice topology and strut morphology on mechanical and biological performance. The thesis was divided into four studies aiming to (1) develop an effective design model for producing structures with biomimetic architecture and biomimetic properties by altering topological characteristics in the lattice, (2) to investigate how topology affects mechanical properties at different scales to help select lattice topologies according to implant dimensions, (3) to see how design parameters from this model affect osteogenesis, and (4) to see how post-processing methods can be applied to enhance mechanical performance and bioactivity. Towards this direction the major findings from each study can be outlined as follows:

- Stochastic lattice structures can be fully geometrically defined using three design parameters: connectivity, strut density and strut thickness. Out of the three design parameters that were used, connectivity was not only a porosity regulator (like strut density and strut thickness), but also a key factor for structure's mechanical behaviour. Increasing connectivity transitioned the structure from a more compliant bending-dominated behaviour to a more rigid stretch-dominated behaviour, accompanied by a significant improvement of their quasi-static mechanical and fatigue performance. More importantly, increasing the connectivity from 4 to 14 increased the fatigue strength by 60% for a fixed relative density. This single integrated model can define a structure to achieve a broad range of stiffness that are found in trabecular and cortical bone, even as a gradient within the same component. To achieve the same with periodic lattices would require different unit cells, with individual regression models for each unit cell used.
- Lattice topological characteristics affect the mechanical properties of lattices depending on aspect ratio (height-to-width ratio). Stiffness is highly affected by aspect ratio and topology owing to different induced failure patterns. A layer-by-layer collapse leading to densification was observed in bend-dominated lattices, while failure through the formation of localised shear bands was observed in the stretch-dominated lattices. By testing lattices of different topologies, it was found that stretch-dominated lattices become stiffer for increasing aspect ratio, while bend-dominated lattices become stiffer for decreasing aspect ratio. Extended mechanical tests also showed that these topology-dependent stiffening mechanisms were not affected by relative density and lattice size. This is important to consider when making engineering components that incorporate lattice structures with low or high aspect ratio, as their actual mechanical properties may be drastically different to coupons used for mechanical characterisation according to international testing standards. Of particular interest are lattice structures integrated into cementless orthopaedic implants and designed to match the stiffness of bone to enable active bone remodelling with limited stress shielding.
- The selection of topological characteristics should not only derive from mechanical requirements, as lattice structures should employ topologies that benefit cell function towards mature bone formation during osteointegration. The effect of each design parameters used in the design model was investigated using in vitro cell cultures. Nodal connectivity and strut density both positively influence cell differentiation, ECM formation and maturation. Lattice structures etched with alkali solution exhibited high hydrophilicity and achieved high cell seeding efficiency even among

designs with diverge surface areas and porosities, contrary to what is usually observed in periodic scaffolds. High porosity (>90%) and large pore size (>1000 μm) promote cell proliferation while suppressing cell differentiation. This suggests that the mature bone tissue formation is not correlated to cell proliferation but rather with the scaffold's internal geometry. High connectivity had the most pronounced effect on osteoblastic differentiation due the increased surface area and number of connections around the nodes. Strut density should be used as parameter to tune pore size. In particular, increasing strut density reduces pore size. Moreover though, pore size is also affected by strut thickness. This is a parameter that has not been investigated in literature extensively. Still, decreasing strut thickness while keeping the other two parameters constant, leads to higher surface area to structure volume ratio that benefits cell-to-cell communication, and eventually accelerates bone matrix formation and mineralisation. A strut thickness between 200 to 300 μm appears ideal for bone replacing lattices as it mimics the trabeculae thickness in natural bone, while it provides high structural integrity without producing overly stiff lattices. Overall, increasing connectivity and adjusting strut density and strut thickness to match the properties of natural bone is a novel technique offering high mechanical endurance, high porosity, and favourable micro-architecture for rapid ossification.

- In the last part of the study, an optimal stochastic lattice design was suggested incorporating a high connectivity ($Z=8$), a middle-ranged strut thickness ($t=260\text{ }\mu\text{m}$), and strut density ($d=8\text{ struts/mm}^3$) adjusted to produce a structure with a pore size of 600 μm which is considered the ideal for osteogenesis. A novel chemical-electrochemical surface treatment was applied to see the effect of surface finish on fatigue strength and osteogenic differentiation of mesenchymal stromal cells when applied to CP-Ti lattices. These post-processes are typically used in medical-grade alloys to smoothen strut surfaces. However, they lead to drastic reduction of strut thickness of $\approx 100\text{ }\mu\text{m}$. When used in CP-Ti lattices, they successfully removed as-built irregularities from strut surface while leading to a moderate strut reduction of $\approx 40\text{ }\mu\text{m}$. This led to a much superior fatigue strength as opposed to an as-built lattice of same strut thickness. Moreover, the surface treatment not only led to smoothening of the structure surface, but also to the introduction of a micro-rough topography that benefited osteogenic differentiation without compromising fatigue strength.

These outcomes suggest that 3D-printed lattice structures made of the ductile CP-Ti can be designed to meet challenging load-bearing requirements, while providing topological cues that benefit mature osteointegration. In addition, CP-Ti lattices can be effectively post-processed for a smoother surface finish that enhances endurance and osteoconduction. Compared to alloy counterparts, this surface treatment can be performed with minimal strut thickness reduction. This is particularly important as fine lattice structures can be additively manufactured with near-net shape even after post-processing.

In conclusion, the presented design approach provides high design freedom and enables the integration of stochastic lattices into large-scale cementless implants with bespoke shape and mechanical properties for improved osseointegration after surgery.

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Appendix

A.1. Laser parameters and assessment of strut morphology

Table A.1. Laser parameters for the additive manufacture of struts					
Data are grouped based on the nominal strut thickness t . Point distance δ_p was constant at 50 μm .					
	Nominal strut thickness t [μm]				
	170	230	260	300	380
Power P [W]	50	50	200	200	200
Strut angle	Exposure time δt [μs] for different strut angles				
18° – 36°	89	321	48	84	176
36° – 54°	131	419	67	120	247
54° – 72°	124	450	75	139	279
72° – 90°	132	438	72	132	265

Table A.2. Measurement of strut thickness using micro-CT				
5 struts were measured for each range of printing angles.				
	Nominal strut thickness t [μm]			
Strut angle θ	170	230	300	380
Kinked struts ($\theta = 25^\circ$)	169.8 $\pm$ 4.8	229.0 $\pm$ 16.1	295.7 $\pm$ 20.1	383.1 $\pm$ 13.0
25° < θ $\leq$ 50°	170.3 $\pm$ 5.1	231.9 $\pm$ 9.8	303.4 $\pm$ 19.6	384.7 $\pm$ 15.2
50° < θ $\leq$ 75°	168.9 $\pm$ 6.6	223.6 $\pm$ 5.2	319.0 $\pm$ 12.3	376.5 $\pm$ 3.7
75° < θ $\leq$ 90°	167.2 $\pm$ 3.5	226.9 $\pm$ 9.6	303.6 $\pm$ 7.6	377.7 $\pm$ 16.7
Population mean $\pm$ SD	169.0 $\pm$ 4.5	227.8 $\pm$ 10.5	305.4 $\pm$ 16.9	380.5 $\pm$ 12.6

The specific enthalpy ΔH [J/kg] was calculated using *Equation 2.11* and all laser parameter sets. Regression models were developed for 4 groups of build angles used. This model allows accurate adjustment of laser parameters for any desired strut thickness – at least within the tested range. The material properties used in *Equation 2.10* are summarised in **Table A.3**.

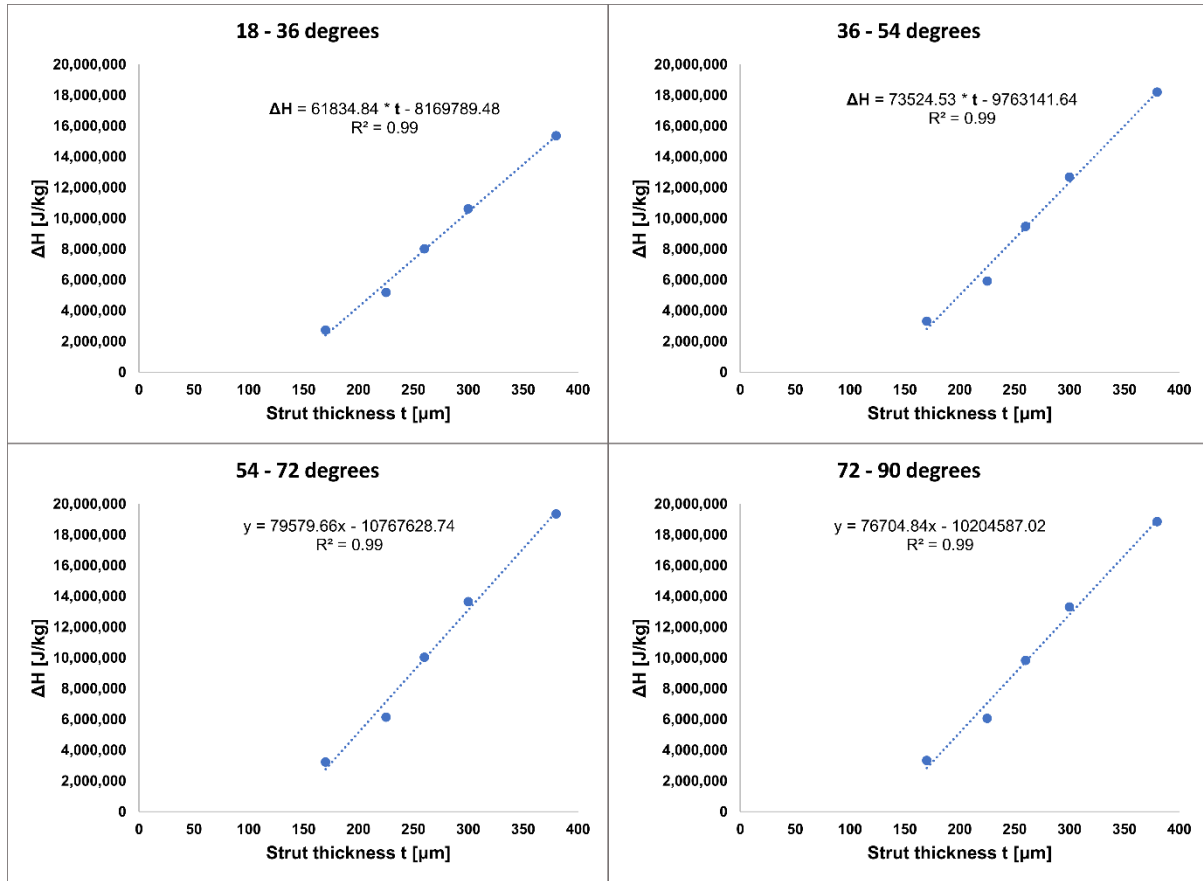


Figure A.1. Regression models for specific enthalpy and resultant strut thickness

Table A3. Properties of solid commercially pure titanium Grade B	
Absorptivity A [-]	0.55
Thermal diffusivity D [m^2/s]	$8.23 \cdot 10^{-6}$
Density ρ_s [kg/m^3]	4510

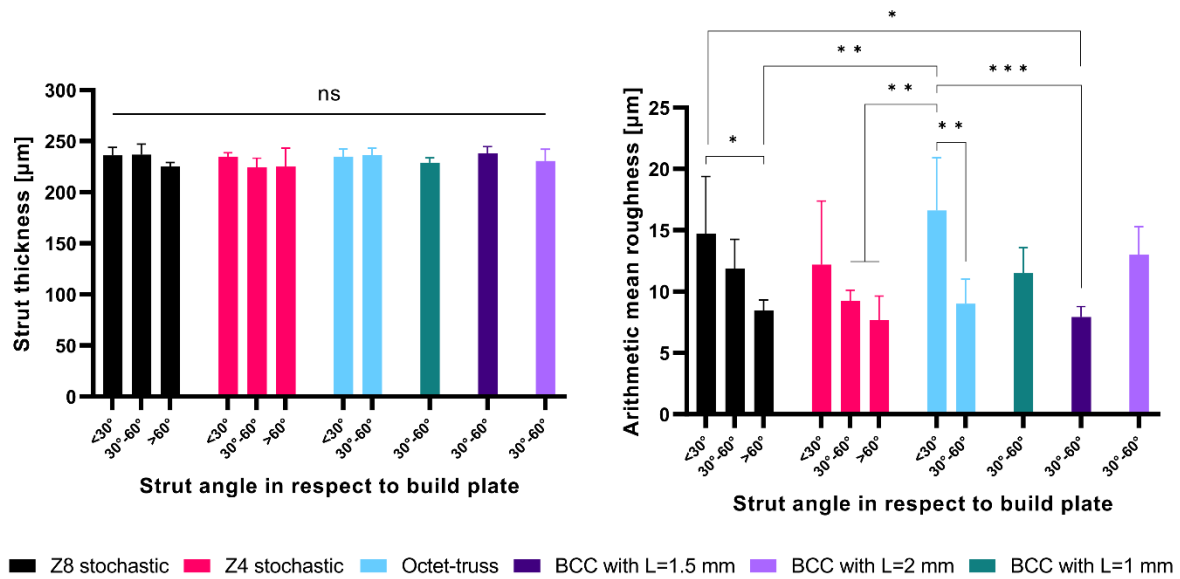


Figure A.2. Measured strut thickness and surface roughness for different lattice topologies. All specimens were manufactured with a nominal strut thickness of 230 μm. No statistical difference was found between lattice types and build angles. Each column represents the mean of n=5 unique struts.

A.2. PCR reaction methodology

PCR reactions were carried out using a Lightcycler 96 PCR instrument (Roche Diagnostics Ltd, UK). PCR amplification reactions were performed using three technical replicates, in a total volume of 10 μL containing 5 μL 2X GoTaq qPCR mastermix, 0.2 μL 50X GoScript reverse transcriptase mix, final concentrations of 250 nM primers and 2 μL of extracted RNA. Cycling parameters for the RT-qPCR assay were as follows: an initial reverse transcription step at 45 °C for 15 min, reverse transcriptase inactivation/initial denaturation at 95 °C for 2 mins, followed by 45 cycles of 95 °C for 15 sec and an annealing temperature of 60°C for β-actin, osteopontin and osteocalcin or 65°C for collagen for 1 min. A no template control consisting of water instead of RNA, was also added in triplicate.

Primer efficiencies were calculated using serial dilutions of synthetic gblock sequence (Integrated DNA Technologies, USA) and this efficiency was used in $\Delta\Delta C_t$ calculations.

A.3. Analytical expressions for the pore size in periodic lattices

Pore size d_p was defined as the diameter of the largest sphere that fits within the polyhedral voids formed by the struts (**Figure A.3**). The orientation of the struts relative to the XY plane is 35.26° in BCC lattices and 0° and 45° in octet-truss lattices.

In BCC lattices, each unit cell consists of a single node with eight struts connected to it. As a result, two consecutive unit cells are required to form a complete octahedral void, which defines the pore size in this topology.

In octet-truss lattices, the unit cell results from the superposition of octahedral and tetrahedral substructures. A single unit cell contains a central octahedral void and eight smaller tetrahedral voids near its edges. Therefore, two distinct pore sizes were calculated:

- The larger pore size, corresponding to the inscribed sphere within the central octahedral void.
- The smaller pore size, corresponding to the inscribed spheres within the tetrahedral voids at the edges.

Partial octahedral voids that form at the interfaces between adjacent unit cells were not considered in this calculation.

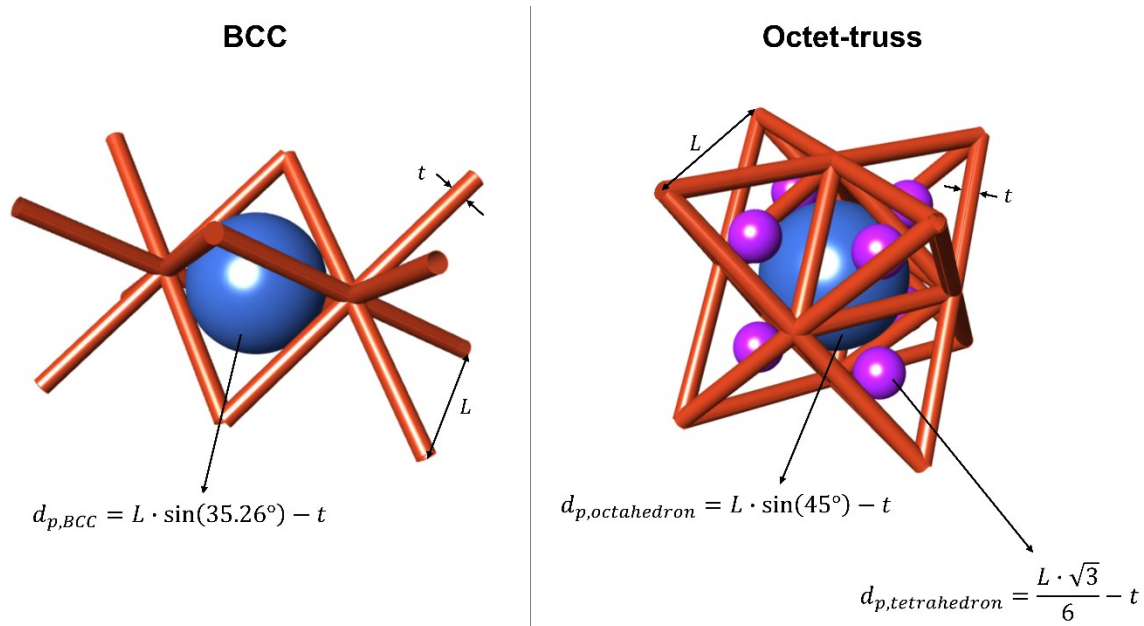


Figure A.3. Analytical calculation of pore sizes in BCC and octet-truss lattice structures.

A.4. Supplementary Figures

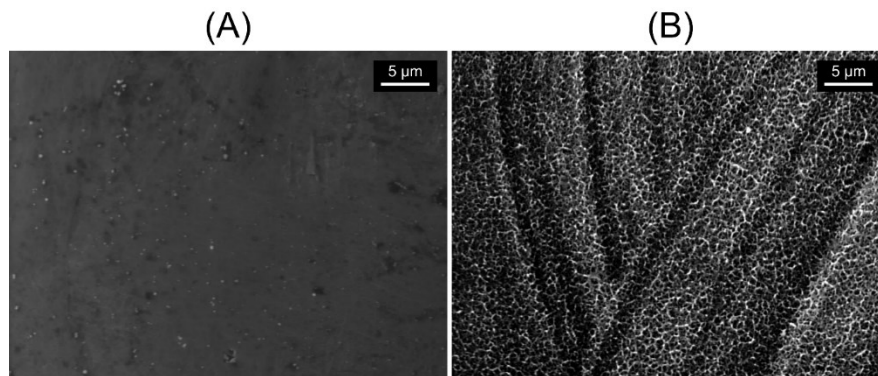


Figure A.4. Effect of alkali treatment on surface topography of pure titanium. SEM images of additively manufactured strut surface (A) before and (B) after alkali treatment with 4M NaOH. Figure reproduced under CC BY license from (121).

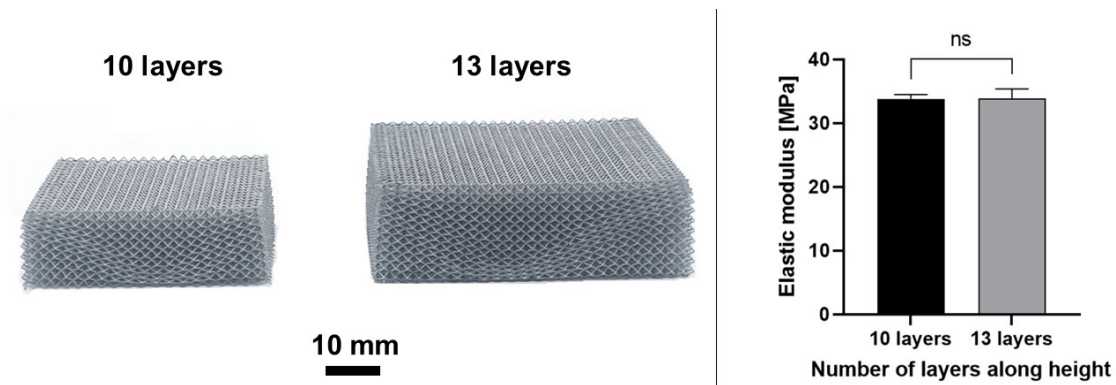


Figure A.5. Compression test results on large BCC lattices designed with 10 and 13 layers. The failure pattern and measured elastic modulus were similar in both cases ($n=5$).

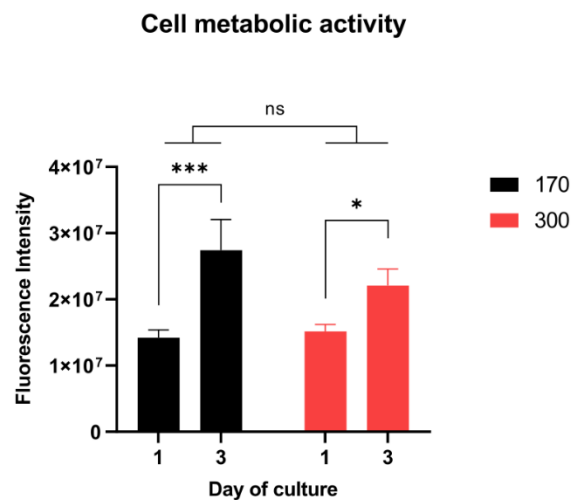


Figure A.6. Cellular metabolic activity in scaffolds with different strut thicknesses. Sample size $n=4$.

A.5. Statistical analyses result summary

A.5.1. The effect of design parameters on the mechanical properties of stochastic structures

Results from the 3-way ANOVA investigating the effect of connectivity Z , strut density d , strut thickness t on the mechanical properties of stochastic lattice structures are provided in the following tables. Tables include the main effect of each parameter and the interaction between parameters. A sample size of $n=5$ per design combination was used in all cases.

Table A.4. The effect of design parameters on the relative density of stochastic structures			
Source of variation	Degrees of freedom	F-value	P-value
Z	3	3366.873	<0.001
d	2	11588.637	<0.001
t	1	4844.490	<0.001
$Z * d$	6	21.361	<0.001
$Z * t$	3	23.777	<0.001
$d * t$	2	109.485	<0.001
$Z * d * t$	6	2.490	0.028

Table A.5. The effect of design parameters on the elastic modulus of stochastic structures			
Source of variation	Degrees of freedom	F-value	P-value
Z	3	1871.574	<0.001
d	2	4783.015	<0.001
t	1	1777.749	<0.001
$Z * d$	6	263.125	<0.001
$Z * t$	3	88.637	<0.001
$d * t$	2	289.252	<0.001
$Z * d * t$	6	10.026	<0.001

Table A.6. The effect of design parameters on the ultimate strength of stochastic structures			
Source of variation	Degrees of freedom	F-value	P-value
<i>Z</i>	3	9226.959	<0.001
<i>d</i>	2	31367.823	<0.001
<i>t</i>	1	10361.551	<0.001
<i>Z * d</i>	6	1688.248	<0.001
<i>Z * t</i>	3	481.142	<0.001
<i>d * t</i>	2	2236.028	<0.001
<i>Z * d * t</i>	6	91.654	<0.001

A.5.2. The effect of aspect ratio on mechanical properties

The results of Tukey's multiple comparison tests for each mechanical property and lattice type are summarised in the following tables. Only significantly different pairs are shown ($\alpha=0.05$). A sample size of $n=5$ per design combination was used in all cases.

Table A.7. Pairwise comparisons of the elastic modulus of the Z8 stochastic lattices		
Compared aspect ratios	Mean difference	P-value
0.50 vs. 2.54	-100.6	<0.001
0.50 vs. 3.00	-188.1	<0.001
0.65 vs. 2.54	-132.4	<0.001
0.65 vs. 3.00	-219.9	<0.001
1.00 vs. 2.54	-112.9	<0.001
1.00 vs. 3.00	-200.4	<0.001
1.62 vs. 2.54	-74.86	0.005
1.62 vs. 3.00	-162.4	<0.001
2.00 vs. 2.54	-84.86	0.001
2.00 vs. 3.00	-172.4	<0.001
2.54 vs. 3.00	-87.50	0.001

Table A.8. Pairwise comparisons of the elastic modulus of BCC lattices		
Compared aspect ratios	Mean difference	P-value
0.50 vs. 0.65	117.8	0.010
0.50 vs. 1.00	271.7	<0.001
0.50 vs. 1.62	316.7	<0.001
0.50 vs. 2.00	257.5	<0.001
0.50 vs. 2.54	250.9	<0.001
0.50 vs. 3.00	293.7	<0.001
0.65 vs. 1.00	153.9	<0.001
0.65 vs. 1.62	198.9	<0.001
0.65 vs. 2.00	139.8	0.002
0.65 vs. 2.54	133.1	0.003
0.65 vs. 3.00	175.9	<0.001

Table A.9. Pairwise comparisons of the yield strength of the Z8 stochastic lattices		
Compared aspect ratios	Mean difference	P-value
0.50 vs. 0.65	0.9720	0.001
0.50 vs. 1.00	0.8060	0.01
0.50 vs. 1.62	0.8020	0.01
0.50 vs. 2.00	0.9660	0.002
0.50 vs. 2.54	0.9900	0.001
0.50 vs. 3.00	0.9800	0.001

Table A.10. Pairwise comparisons of the yield strength of BCC lattices		
Compared aspect ratios	Mean difference	P-value
0.50 vs. 0.65	1.266	0.04
0.50 vs. 1.00	1.776	0.002
0.50 vs. 1.62	1.816	0.001
0.50 vs. 2.00	2.094	<0.001
0.50 vs. 2.54	2.250	<0.001
0.50 vs. 3.00	2.020	<0.001

Table A.11. Pairwise comparisons of the ultimate strength of the Z8 stochastic lattices		
Compared aspect ratios	Mean difference	P-value
0.50 vs. 0.65	0.8570	<0.001
0.50 vs. 1.00	0.9022	<0.001
0.50 vs. 1.62	0.9278	<0.001
0.50 vs. 2.00	1.033	<0.001
0.50 vs. 2.54	0.8624	<0.001
0.50 vs. 3.00	0.7578	<0.001

Table A.12. Pairwise comparisons of the ultimate strength of BCC lattices		
Compared aspect ratios	Mean difference	P-value
0.50 vs. 1.00	2.025	<0.001
0.50 vs. 1.62	2.189	<0.001
0.50 vs. 2.00	1.744	<0.001
0.50 vs. 2.54	1.821	<0.001
0.50 vs. 3.00	2.129	<0.001
0.65 vs. 1.00	1.542	<0.001
0.65 vs. 1.62	1.706	<0.001
0.65 vs. 2.00	1.261	<0.001
0.65 vs. 2.54	1.338	<0.001
0.65 vs. 3.00	1.646	<0.001

Table A.13. Pairwise comparisons of the elastic modulus of octet-truss lattices		
Compared aspect ratios	Mean difference	P-value
0.50 vs. 1.62	-536.7	0.003
0.50 vs. 3.00	-837.4	<0.001

Table A.14. Pairwise comparisons of the ultimate strength of octet-truss lattices		
Compared aspect ratios	Mean difference	P-value
0.50 vs. 1.62	9.518	<0.001
0.50 vs. 3.00	11.44	<0.001
1.62 vs. 3.00	1.926	0.03

Table A.15. Pairwise comparisons of the elastic modulus of the Z4 stochastic structures		
Compared aspect ratios	Mean difference	P-value
0.50 vs. 1.62	15.96	<0.001
0.50 vs. 3.00	13.87	<0.001
1.62 vs. 3.00	-2.082	0.67

Table A.16. Pairwise comparisons of the ultimate strength of the Z4 stochastic structures		
Compared aspect ratios	Mean difference	P-value
0.50 vs. 1.62	0.4080	<0.001
0.50 vs. 3.00	0.7420	<0.001
1.62 vs. 3.00	0.3340	<0.001

2-way ANOVA was used to investigate the effect of aspect ratio and unit cell size on the mechanical properties of BCC lattices.

Table A.17. Effect of aspect ratio and unit cell size on the elastic modulus of BCC lattices			
Source of variation	Degree of Freedom	F-value	P-value
Aspect ratio * Unit cell size	2	F (2, 24) = 38.60	<0.001
Aspect ratio	1	F (1, 24) = 10750	<0.001
Unit cell size	2	F (2, 24) = 38.98	<0.001

Table A.18. Effect of aspect ratio and unit cell size on the compressive strength of BCC lattices			
Source of variation	Degree of Freedom	F-value	P-value
Aspect ratio * Unit cell size	2	F (2, 24) = 59.34	<0.001
Aspect ratio	1	F (1, 24) = 20654	<0.001
Unit cell size	2	F (2, 24) = 137.7	<0.001

Table A.19. Effect of aspect ratio and unit cell size on the energy absorption of BCC lattices			
Source of variation	Degree of Freedom	F-value	P-value
Aspect ratio * Unit cell size	4	F (4, 36) = 39.50	<0.001
Aspect ratio	2	F (2, 36) = 7796	<0.001
Unit cell size	2	F (2, 36) = 50.65	<0.001

2-way ANOVA was used to investigate the effect of aspect ratio and number of unit cells along the shortest edge on the mechanical properties of periodic lattices.

Table A.20. Effect of aspect ratio and number of unit cells on the elastic modulus of octet-truss lattices			
Source of variation	Degree of Freedom	F-value	P-value
Aspect ratio * No. unit cells	4	F (4, 24) = 23.26	<0.001
Aspect ratio	2	F (2, 24) = 20.84	<0.001
No. of unit cells	2	F (2, 24) = 29.22	<0.001

Table A.21. Effect of aspect ratio and number of unit cells on the elastic modulus of BCC lattices			
Source of variation	Degree of Freedom	F-value	P-value
Aspect ratio * No. unit cells	4	F (4, 24) = 64.91	<0.001
Aspect ratio	2	F (2, 24) = 18.52	<0.001
No. unit cells	2	F (2, 24) = 180.4	<0.001

Table A.22. Effect of aspect ratio and number of unit cells on the compressive strength of octet-truss lattices			
Source of variation	Degree of Freedom	F-value	P-value
Aspect ratio * No. unit cells	4	F (4, 24) = 4.852	0.005
Aspect ratio	2	F (2, 24) = 317.7	<0.001
No. unit cells	2	F (2, 24) = 5.128	0.01

Table A.23. Effect of aspect ratio and number of unit cells on the compressive strength of BCC lattices			
Source of variation	Degree of Freedom	F-value	P-value
Aspect ratio * No. unit cells	4	F (4, 24) = 5.158	0.004
Aspect ratio	2	F (2, 24) = 185.0	<0.001
No. of unit cells	2	F (2, 24) = 195.4	<0.001

A.5.3. The effect of lattice design parameters on osteogenesis

Results from 2-way or 3-way ANOVA investigating the effect of connectivity Z , strut density d and day of culture on cell adhesion, cell proliferation, and the expression levels of key biomarkers are provided in the following tables. Tables include the main effect of each parameter and the interaction between parameters. A sample size of $n=3$ per scaffold design and day was used in all cases.

Table A.24. Effect connectivity and strut density on cell adhesion after seeding			
Source of Variation	Degrees of freedom	F-value	P-value
Z	1	F (1, 8) = 0.02192	0.10
d	1	F (1, 8) = 3.342	0.12
Z × d	1	F (1, 8) = 3.056	0.89

Table A.25. Effect of connectivity, strut density and day of culture on proliferation rate			
Source of Variation	Degrees of freedom	F-value	P-value
Day	2	F (2, 24) = 6.682	0.005
Z	1	F (1, 24) = 20.02	<0.001
d	1	F (1, 24) = 27.80	<0.001
Day × Z	2	F (2, 24) = 2.070	0.15
Day × d	2	F (2, 24) = 1.421	0.26
Z × d	1	F (1, 24) = 5.785	0.02
Day × Z × d	2	F (2, 24) = 1.271	0.30

Table A.26. Effect of connectivity, strut density and day of culture on picosirius red content			
Source of Variation	Degrees of freedom	F-value	P-value
Day	1	F (1, 16) = 304.8	<0.001
Z	1	F (1, 16) = 10.08	0.006
d	1	F (1, 16) = 24.96	<0.001
Day × Z	1	F (1, 16) = 0.8452	0.37
Day × d	1	F (1, 16) = 1.479	0.24
Z × d	1	F (1, 16) = 3.324	0.09
Day × Z × d	1	F (1, 16) = 6.785	0.02

Table A.27. Effect of connectivity, strut density and day of culture on ALP expression			
Source of Variation	Degrees of freedom	F-value	P-value
Day	3	F (3, 32) = 204.9	<0.001
Z	1	F (1, 32) = 2.355	0.13
d	1	F (1, 32) = 43.79	<0.001
Day × Z	3	F (3, 32) = 0.5339	0.66
Day × d	3	F (3, 32) = 24.36	<0.001
Z × d	1	F (1, 32) = 0.07433	0.79
Day × Z × d	3	F (3, 32) = 8.686	<0.001

Table A.28. Effect of connectivity, strut density and day of culture on collagen-I expression			
Source of Variation	Degrees of freedom	F-value	P-value
Day	1	F (1, 16) = 118.4	<0.001
Z	1	F (1, 16) = 20.67	<0.001
d	1	F (1, 16) = 4.683	0.05
Day × Z	1	F (1, 16) = 13.85	0.002
Day × d	1	F (1, 16) = 9.797	0.006
Z × d	1	F (1, 16) = 0.1340	0.72
Day × Z × d	1	F (1, 16) = 0.8591	0.37

Table A.29. Effect of connectivity, strut density and day of culture on osteopontin expression			
Source of Variation	Degrees of freedom	F-value	P-value
Day	1	F (1, 16) = 219.5	<0.001
Z	1	F (1, 16) = 28.44	<0.001
d	1	F (1, 16) = 0.5199	0.48
Day × Z	1	F (1, 16) = 26.45	<0.001
Day × d	1	F (1, 16) = 0.4386	0.52
Z × d	1	F (1, 16) = 1.211	0.29
Day × Z × d	1	F (1, 16) = 1.162	0.30

Table A.30. Effect of connectivity, strut density and day of culture on osteocalcin expression			
Source of Variation	Degrees of freedom	F-value	P-value
Day	1	F (1, 16) = 137.9	<0.001
Z	1	F (1, 16) = 12.61	0.003
d	1	F (1, 16) = 2.762	0.12
Day × Z	1	F (1, 16) = 6.644	0.02
Day × d	1	F (1, 16) = 0.5382	0.47
Z × d	1	F (1, 16) = 4.990	0.04
Day × Z × d	1	F (1, 16) = 1.431	0.25

A.5.4. The effect of surface treatment on osteogenesis

Table A.31. Effect of surface treatment and day of culture on cell viability			
Source of Variation	Degrees of freedom	F-value	P-value
Treatment	1	$F(1, 12) = 1.577$	0.23
Day	2	$F(2, 12) = 13.56$	$P < 0.001$
Treatment $\times$ Day	2	$F(2, 12) = 0.09960$	0.91

Table A.32. Effect of surface treatment and day of culture on ALP activity			
Source of Variation	Degrees of freedom	F-value	P-value
Treatment	1	$F(1, 12) = 54.89$	$P < 0.001$
Day	2	$F(2, 12) = 40.45$	$P < 0.001$
Treatment $\times$ Day	2	$F(2, 12) = 6.226$	$P = 0.01$